COMPOUNDS AND METHODS FOR MODULATING CELL ADHESION

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CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/021,612, filed on July 12, 1996.

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TECHNICAL FIELD

The present invention relates generally to methods for modulating cell adhesion, and more particularly to cyclic peptides comprising a cadherin cell adhesion recognition sequence, and to the use of such cyclic peptides for inhibiting or enhancing cadherin-mediated cell adhesion.

BACKGROUND OF THE INVENTION

Cell adhesion is a complex process that is important for maintaining tissue integrity and generating physical and permeability barriers within the body. All tissues are divided into discrete compartments, each of which is composed of a specific cell type that adheres to similar cell types. Such adhesion triggers the formation of intercellular junctions (i.e., readily definable contact sites on the surfaces of adjacent cells that are adhering to one another), also known as tight junctions, gap junctions and belt desmosomes. The formation of such junctions gives rise to physical and permeability barriers that restrict the free passage of cells and other biological substances from one tissue compartment to another. For example, the blood vessels of all tissues are composed of endothelial cells. In order for components in the blood to enter a given tissue compartment, they must first pass from the lumen of a blood vessel through the barrier formed by the endothelial cells of that vessel. Similarly, in order for substances to enter the body via the gut, the substances must first pass through a barrier formed by the epithelial cells of that tissue. To enter the blood via the skin, both epithelial and endothelial cell layers must be crossed.

Cell adhesion is mediated by specific cell surface adhesion molecules (CAMs). There are many different families of CAMs, including the immunoglobulin, integrin, selectin and cadherin superfamilies, and each cell type expresses a unique combination of these molecules. Cadherins are a rapidly expanding family of calcium-

5   dependent CAMs (Munro et al., *In: Cell Adhesion and Invasion in Cancer Metastasis*, P. Brodt, ed., pp. 17-34, RG Landes Co.(Austin TX, 1996). The classical cadherins (abbreviated CADs) are integral membrane glycoproteins that generally promote cell adhesion through homophilic interactions (a CAD on the surface of one cell binds to an identical CAD on the surface of another cell), although CADs also appear to be capable

10   of forming heterotypic complexes with one another under certain circumstances and with lower affinity. Cadherins have been shown to regulate epithelial, endothelial, neural and cancer cell adhesion, with different CADs expressed on different cell types. N (neural) - cadherin is predominantly expressed by neural cells, endothelial cells and a variety of cancer cell types. E (epithelial) - cadherin is predominantly expressed by

15   epithelial cells. Other CADs are P (placental) - cadherin, which is found in human skin and R (retinal) - cadherin. A detailed discussion of the classical cadherins is provided in Munro SB et al., 1996, *In: Cell Adhesion and Invasion in Cancer Metastasis*, P. Brodt, ed., pp.17-34 (RG Landes Company, Austin TX).

The structures of the CADs are generally similar. As illustrated in

20   Figure 1, CADs are composed of five extracellular domains (EC1-EC5), a single hydrophobic domain (TM) that transverses the plasma membrane (PM), and two cytoplasmic domains (CP1 and CP2). The calcium binding motifs DXNDN (SEQ ID NO:41), DXD and LDRE (SEQ ID NO:40) are interspersed throughout the extracellular domains. The first extracellular domain (EC1) contains the classical cadherin cell

25   adhesion recognition (CAR) sequence, HAV (His-Ala-Val), along with flanking sequences on either side of the CAR sequence that may play a role in conferring specificity. Synthetic peptides containing the CAR sequence and antibodies directed against the CAR sequence have been shown to inhibit CAD-dependent processes (Munro et al., *supra*; Blaschuk et al., *J. Mol. Biol. 211*:679-82, 1990; Blaschuk et al.,

30   *Develop. Biol. 139*:227-29, 1990; Alexander et al., *J. Cell. Physiol. 156*:610-18, 1993). The three-dimensional solution and crystal structures of the EC1 domain have been

determined (Overduin et al., *Science* *267*:386-389, 1995; Shapiro et al., *Nature* *374*:327-337, 1995).

Although cell adhesion is required for certain normal physiological functions, there are situations in which the level of cell adhesion is undesirable. For example, many pathologies (such as autoimmune diseases, cancer and inflammatory diseases) involve abnormal cellular adhesion. Cell adhesion may also play a role in graft rejection. In such circumstances, modulation of cell adhesion may be desirable.

In addition, permeability barriers arising from cell adhesion create difficulties for the delivery of drugs to specific tissues and tumors within the body. For example, skin patches are a convenient tool for administering drugs through the skin. However, the use of skin patches has been limited to small, hydrophobic molecules because of the epithelial and endothelial cell barriers. Similarly, endothelial cells render the blood capillaries largely impermeable to drugs, and the blood/brain barrier has hampered the targeting of drugs to the central nervous system. In addition, many solid tumors develop internal barriers that limit the delivery of anti-tumor drugs and antibodies to inner cells.

Attempts to facilitate the passage of drugs across such barriers generally rely on specific receptors or carrier proteins that transport molecules across barriers *in vivo*. However, such methods are often inefficient, due to low endogenous transport rates or to the poor functioning of a carrier protein with drugs. While improved efficiency has been achieved using a variety of chemical agents that disrupt cell adhesion, such agents are typically associated with undesirable side-effects, may require invasive procedures for administration and may result in irreversible effects. It has been suggested that linear synthetic peptides containing a cadherin CAR sequence may be employed for drug transport (WO 91/04745), but such peptides are often metabolically unstable and are generally considered to be poor therapeutic agents.

Accordingly, there is a need in the art for compounds that modulate cell adhesion and improve drug delivery across permeability barriers without such disadvantages. The present invention fulfills this need and further provides other related advantages.

SUMMARY OF THE INVENTION

The present invention provides cyclic peptides and methods for modulating cadherin-mediated cell adhesion. Within one aspect, the present invention provides cyclic peptides comprising the sequence His-Ala-Val, wherein the cyclic peptides modulate cadherin-mediated cell adhesion. Within one embodiment a cyclic peptide has the formula:

$$(Z_1)\text{-}(Y_1)\text{-}(X_1)\text{-His-Ala-Val-}(X_2)\text{-}(Y_2)\text{-}(Z_2)$$
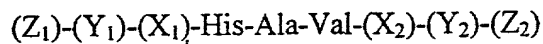
wherein X_1, and X_2 are optional, and if present, are independently selected from the group consisting of amino acid residues and combinations thereof in which the residues are linked by peptide bonds, and wherein X_1 and X_2 independently range in size from 0 to 10 residues, such that the sum of residues contained within X_1 and X_2 ranges from 1 to 12; wherein Y_1 and Y_2 are independently selected from the group consisting of amino acid residues, and wherein a covalent bond is formed between residues Y_1 and Y_2; and wherein Z_1 and Z_2 are optional, and if present, are independently selected from the group consisting of amino acid residues and combinations thereof in which the residues are linked by peptide bonds. Such cyclic peptides may comprise modifications such as an N-acetyl or N-alkoxybenzyl group and/or a C-terminal amide or ester group. Cyclic peptides may be cyclized via, for example, a disulfide bond; an amide bond between terminal functional groups, between residue side-chains or between one terminal functional group and one residue side chain; a thioether bond or $\delta_1\delta_1$-ditryptophan, or a derivative thereof. Cyclic peptides may further be linked to a targeting agent, a drug, a solid support and/or a detectable marker.

Within another aspect, the present invention provides pharmaceutical compositions comprising one or more cyclic peptides as described above in combination with a pharmaceutically acceptable carrier. Such compositions may further comprise a drug.

Within further aspects, methods are provided for modulating cell adhesion, comprising contacting a cadherin-expressing cell with a cyclic peptide as described above.

Within one such aspect, methods are provided for reducing unwanted

5   cellular adhesions in a mammal, comprising administering to a mammal a cyclic peptide as described above.

In another aspect, the present invention provides methods for enhancing the delivery of a drug through the skin of a mammal, comprising contacting epithelial cells of a mammal with a cyclic peptide as described above and a drug under conditions

10   and for a time sufficient to allow passage of the drug across the epithelial cells.

In a further aspect, a method is provided for enhancing the delivery of a drug to a tumor in a mammal, comprising administering to a mammal a pharmaceutical composition comprising a cyclic peptide as described above and a drug.

Within related aspects, methods for treating cancer and/or inhibiting

15   metastasis of tumor cells in a mammal are provided, comprising administering to a mammal afflicted with cancer a cyclic peptide as described above.

In a further aspect, methods are provided for inducing apoptosis in a cadherin-expressing cell, comprising contacting a cadherin-expressing cell with a cyclic peptide as described above.

20   The present invention also provides, within other aspects, methods for inhibiting angiogenesis in a mammal, comprising administering to a mammal a cyclic peptide as described above.

Within a further embodiment, the present invention provides methods for enhancing drug delivery to the brain of a mammal, comprising administering to a

25   mammal a cyclic peptide as described above.

In still further aspects, methods are provided for enhancing cell adhesion. Within one such aspect, methods for enhancing wound healing in a mammal are provided, comprising contacting a wound in a mammal with a cyclic peptide as described above.

30   Within a related aspect, the present invention provides methods for enhancing adhesion of foreign tissue implanted within a mammal, comprising

contacting a site of implantation of foreign tissue in a mammal with a cyclic peptide as described above.

The present invention also provides, in further aspects, methods for enhancing neurite outgrowth, comprising contacting a neuron with a cyclic peptide as described above.

Within a related aspect, methods for treating spinal cord injuries in a mammal are provided, comprising administering to a mammal a cyclic peptide as described above.

In a further related aspect, the present invention provides methods for treating a demyelinating neurological disease in a mammal, comprising administering to a mammal a cyclic peptide as described above.

The present invention also provides methods for modulating the immune system of a mammal, comprising administering to a mammal a cyclic peptide as described above.

In yet another aspect, methods for preventing pregnancy in a mammal are provided, comprising administering to a mammal a composition as described above.

Within a further aspect, methods are provided for increasing vasopermeability in a mammal, comprising administering to a mammal a cyclic peptide as described above.

The present invention also provides methods for identifying a cyclic peptide capable of modulating cadherin-mediated cell adhesion. One such method comprises: (a) culturing neurons on a monolayer of cells that express N-cadherin in the presence and absence of a candidate cyclic peptide, under conditions and for a time sufficient to allow neurite outgrowth; (b) determining a mean neurite length for the neurons; and (c) comparing the mean neurite length for neurons cultured in the presence of candidate cyclic peptide to the neurite length for neurons cultured in the absence of candidate cyclic peptide.

Within another embodiment, the method comprises: (a) culturing cells that express a cadherin in the presence and absence of a candidate cyclic peptide, under conditions and for a time sufficient to allow cell adhesion; and (b) visually evaluating the extent of cell adhesion among the cells.

In yet another embodiment, the method comprises: (a) culturing NRK cells in the presence and absence of a candidate cyclic peptide, under conditions and for a time sufficient to allow cell adhesion; and (b) comparing the level of cell surface E-cadherin for cells cultured in the presence of candidate cyclic peptide to the level for cells cultured in the absence of candidate cyclic peptide.

In a further embodiment, the method comprises: (a) contacting an epithelial surface of skin with a test marker in the presence and absence of candidate cyclic peptide; and (b) comparing the amount of test marker that passes through the skin in the presence of candidate cyclic peptide to the amount that passes through the skin in the absence of candidate cyclic peptide.

Within another embodiment, the method comprises: (a) contacting a blood vessel with a candidate cyclic peptide; and (b) comparing the extent of angiogenesis of the blood vessel to a predetermined extent of angiogenesis observed for a blood vessel in the absence of candidate cyclic peptide, and therefrom identifying a cyclic peptide capable of modulating cell adhesion.

The present invention also provides, within a further aspect, a kit for administering a drug via the skin of a mammal, comprising: (a) a skin patch; and (b) a cyclic peptide as described above.

Within still further aspects, the present invention provides methods for modulating cell adhesion, comprising contacting a cadherin-expressing cell with an antibody that binds to a cyclic peptide as described above. In one such aspect, a method for targeting a drug to a cadherin-expressing cell in a mammal is provided, comprising administering to a mammal an antibody that binds to a cyclic peptide as described above, wherein the antibody is linked to a drug.

The present invention also provides methods for detecting the presence of cadherin-expressing cells in a sample, comprising: (a) contacting a sample with an antibody that binds to a cyclic peptide as described above under conditions and for a time sufficient to allow formation of an antibody-cadherin complex; and (b) detecting the level of antibody-cadherin complex.

Within another aspect, the present invention provides kits for detecting the presence of cadherin-expressing cells in a sample, comprising: (a) an antibody that binds to a cyclic peptide as described above; and (b) a detection reagent.

These and other aspects of the invention will become evident upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each were individually noted for incorporation.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram depicting the structure of classical CADs. The five extracellular domains are designated EC1-EC5, the hydrophobic domain that transverses the plasma membrane (PM) is represented by TM, and the two cytoplasmic domains are represented by CP1 and CP2. The calcium binding motifs are shown by DXNDN (SEQ ID NO:41), DXD and LDRE (SEQ ID NO:40). The CAR sequence, HAV, is shown within EC1. Cytoplasmic proteins β-catenin (β), α-catenin (α) and α-actinin (ACT), which mediate the interaction between CADs and microfilaments (MF) are also shown.

Figure 2 provides the amino acid sequences of mammalian classical cadherin EC1 domains: human N-cadherin (SEQ ID NO:1), mouse N-cadherin (SEQ ID NO:2), cow N-cadherin (SEQ ID NO:3), human P-cadherin (SEQ ID NO:4), mouse P-cadherin (SEQ ID NO:5), human E-cadherin (SEQ ID NO:6) and mouse E-cadherin (SEQ ID NO:7).

Figure 3 provides the structures of representative cyclic peptides of the present invention (structures on the left hand side), along with similar, but inactive, structures (on the right).

Figure 4 is a histogram depicting the mean neurite length in microns for neurons grown on a monolayer of untransfected 3T3 cells (first column) or 3T3 cells transfected with cDNA encoding N-cadherin (columns 2-4). In the third column, the mean neurite length in the presence of the representative cyclic peptide N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8) is shown. Column 4 depicts the mean neurite length in the presence of the control peptide N-Ac-CHGVC-NH$_2$ (SEQ ID NO:9).

Figure 5 is a graph showing a dose response curve for the representative cyclic peptide N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8) on control 3T3 cells (open circles) and on 3T3 cells expressing N-cadherin (solid circles).

Figure 6 is a histogram depicting the mean neurite length in microns for

5   neurons grown in the presence (solid bars) or absence (cross-hatched bars) of 500 µg/mL of the representative cyclic peptide N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8). In the first pair of bars, neurons were grown on a monolayer of untransfected 3T3 cells. In the remaining columns, the mean neurite length is shown for neurons cultured on 3T3 cells transfected with cDNA encoding N-CAM (second pair of bars), L1 (third pair of bars)

10 or N-cadherin (fourth pair of bars).

Figures 7A-C are photographs showing monolayer cultures of bovine endothelial cells in the presence (Figure 7A) and absence (Figure 7C) of a representative cyclic peptide or in the presence of an inactive control peptide (Figure 7B). Figure 7A shows the cells 30 minutes after exposure to 500 µg/mL N-Ac-CHAVC-NH$_2$ (SEQ ID

15 NO:8). Figure 7B shows the cells 30 minutes after exposure to the control peptide N-Ac-CHGVC-NH$_2$ (SEQ ID NO:9). Figure 7C shows the cells in the absence of cyclic peptide. Note that the endothelial cells retracted from one another in the presence of N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8).

Figures 8A-C are photographs showing monolayer cultures of bovine

20 endothelial cells in the presence (Figure 8A) and absence (Figure 8C) of a representative cyclic peptide or in the presence of an inactive control peptide (Figure 8B). Figure 8A shows the cells 30 minutes after exposure to 500 µg/mL N-Ac-CAHAVDIC-NH$_2$ (SEQ ID NO:10). Figure 8B shows the cells 30 minutes after exposure to the control peptide N-Ac-CAHGVDIC-NH$_2$ (SEQ ID NO:11). Figure 8C shows the cells in the absence of

25 cyclic peptide. In this case, neither of the cyclic peptides show activity.

Figures 9A-C are photographs showing monolayer cultures of bovine endothelial cells in the presence (Figure 9A) and absence (Figure 9C) of a representative cyclic peptide or in the presence of an inactive control peptide (Figure 9B). Figure 9A shows the cells 30 minutes after exposure to 500 µg/mL N-Ac-CAHAVDC-NH$_2$ (SEQ

30 ID NO:16). Figure 9B shows the cells 30 minutes after exposure to the control peptide N-Ac-CAHGVDC-NH$_2$ (SEQ ID NO:17). Figure 9C shows the cells in the absence of

cyclic peptide. Note that the endothelial cells retracted from one another in the presence of N-Ac-CAHAVDC-NH₂ (SEQ ID NO:16).

Figures 10A-C are photographs showing monolayer cultures of bovine endothelial cells in the presence (Figure 10A) and absence (Figure 10C) of a representative cyclic peptide or in the presence of an inactive control peptide (Figure 10B). Figure 10A shows the cells 30 minutes after exposure to 500 µg/mL N-Ac-CSHAVSSC-NH₂ (SEQ ID NO:18). Figure 10B shows the cells 30 minutes after exposure to the control peptide N-Ac-CSHGVSSC-NH₂ (SEQ ID NO:19). Figure 10C shows the cells in the absence of cyclic peptide. Note that the endothelial cells retracted from one another and round up in the presence of N-Ac-CSHAVSSC-NH₂ (SEQ ID NO:18).

Figures 11A-F are photographs showing monolayer cultures of human ovarian cancer cells (SKOV3) in the presence (Figures 11A and D-F) and absence (Figure 11C) of a representative cyclic peptide or in the presence of an inactive control peptide (Figure 11B). Figure 11A shows the cells 24 hours after being cultured in the presence of 500 µg/mL N-Ac-CHAVC-NH₂ (10X magnification). Figure 11B shows the cells (10X magnification) 24 hours after being cultured in the presence of the control peptide N-Ac-CHGVC-NH₂ (SEQ ID NO:9). Figure 11C shows the cells (10X magnification) in the absence of cyclic peptide. Figures 11D-F show the cells (20X magnification) 48 hours after exposure to N-Ac-CHAVC-NH₂ (SEQ ID NO:8) at concentrations of 1 mg/mL, 100 µg/mL and 10 µg/mL, respectively. Note that the SKOV3 cells retract from one another and round-up when cultured in the presence of either 0.5 or 1mg/ml N-Ac-CHAVC-NH₂ (SEQ ID NO:8).

Figures 12A and 12B are photographs showing monolayer cultures of human ovarian cancer cells (SKOV3) 24 hours after exposure to 500µg/mL of the representative cyclic peptide N-Ac-CHAVC-NH₂ (SEQ ID NO:8) (Figure 12A) or the control peptide N-Ac-CHGVC-NH₂ (Figure 12B). Note that the SKOV3 cells round-up when cultured in the presence of 0.5 mg/ml N-Ac-CHAVC-NH₂ (SEQ ID NO:8).

Figures 13A-D are photographs of monolayer cultures of normal rat kidney (NRK) cells untreated (Figure 13A) or after 48 hours of exposure to 1 mg/mL H-CHAVSC-OH (SEQ ID NO:14) (Figure 13B), the control peptide N-Ac-CHGVC-NH₂

(SEQ ID NO:9), (Figure 13C) or the representative cyclic peptide N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8), (Figure 13D). Note that NRK cells retract from one another when cultured in the presence of N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8). Furthermore the NRK cells do not form cobblestone-like monolayers when exposed to this peptide.

5   Figures 14A-D are immunofluorescence photographs of the monolayer normal rat kidney (NRK) cultures shown in Figures 13A-D immunolabeled for E-cadherin. Figure 14A shows untreated cells and Figures 14B-D show cells after 48 hours of exposure to either 1 mg/mL H-CHAVSC-OH (SEQ ID NO:14) (Figure 14B), the control peptide N-Ac-CHGVC-NH$_2$ (SEQ ID NO:9), (Figure 14C) or the

10   representative cyclic peptide N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8), (Figure 14D). Note that E-cadherin expression is greatly reduced in the cells treated with N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8), as compared to the E-cadherin levels expressed by untreated cells and cells treated with the other two cyclic peptides

          Figures 15A-C are photographs showing monolayer cultures of human

15   ovarian cancer cells (OVCAR3) in the presence of varying concentrations of a representative cyclic peptide. Figure 15A shows the cells 24 hours after being cultured in the presence of 1 mg/ml of N-Ac-CHAVSC-NH$_2$ (SEQ ID NO:14). Figure 15B shows the cells 24 hours after being cultured in the presence of 100 µg/ml of N-Ac-CHAVSC-NH$_2$ (SEQ ID NO:14). Figure 15C shows the cells 24 hours after being

20   cultured in the presence of 10 µg/ml of N-Ac-CHAVSC-NH$_2$ (SEQ ID NO:14). Note that the cells retract form one another in the presence of 100 µg/ml of N-Ac-CHAVSC-NH$_2$ (SEQ ID NO:14), whereas they round up in the presence of 1 mg/ml of this peptide.

          Figures 16A and B are photographs showing cultures of human

25   melanoma ME115 cells in the presence (Figure 16B) and absence (Figure 16A) of a representative cyclic peptide. The cells have been immunolabeled for cadherin. Figure 16B shows the cells 48 hours after being cultured in the presence of 500 µg/ml of N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8). Figure 16A shows untreated cultures of human melanoma ME115 cells. Note that cadherin is localized in intracellular vesicles in cells

30   treated with peptide, whereas it is present at the surface in the untreated cells.

Figures 17A and B are photographs showing monolayer cultures of A1N4 human breast epithelial cells in the presence (Figure 17B) and absence (Figure 17A) of a representative cyclic peptide. The cells have been immunolabeled for E-cadherin. Figure 17B shows the cells 48 hours after being cultured in the presence of

5   500 µg/ml of N-Ac-CHAVC-NH₂ (SEQ ID NO:8). Figure 17A shows untreated monolayer cultures of A1N4 human breast epithelial cells. Note that the distribution of E-cadherin is non-contiguous in cells treated with the cyclic peptide. Furthermore, gaps have appeared in the monolayer of cells treated with the peptide.

10   DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention provides cyclic peptides capable of modulating cadherin-mediated cell adhesion. Certain cyclic peptides described herein inhibit cell adhesion. Such cyclic peptides may generally be used, for example, to treat diseases or other conditions characterized by undesirable cell adhesion or to facilitate

15   drug delivery to a specific tissue or tumor. Alternatively, a cyclic peptide may, such as when linked to a matrix or to another cyclic peptide via a linker, be used to enhance cell adhesion. Such cyclic peptide-matrix conjugates may be used, for example, to improve cell adhesion (e.g., to supplement or replace stitches or to facilitate wound healing) or to enhance or direct neurite outgrowth.

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CYCLIC PEPTIDES

The term "cyclic peptide," as used herein, refers to a peptide or salt thereof that comprises (1) an intramolecular covalent bond between two non-adjacent residues and (2) at least one cadherin cell adhesion recognition (CAR) sequence. The

25   intramolecular bond may be a backbone to backbone, side-chain to backbone or side-chain to side-chain bond (i.e., terminal functional groups of a linear peptide and/or side chain functional groups of a terminal or interior residue may be linked to achieve cyclization). Preferred intramolecular bonds include, but are not limited to, disulfide, amide and thioether bonds. At least one CAR sequence generally comprises HAV (His-

30   Ala-Val). Cyclic peptides may contain only one CAR sequence, or may additionally contain one or more other adhesion molecule binding sites, which may or may not be

CARs. Such additional sequences may be separated by a linker (*i.e.*, one or more peptides not derived from a CAR sequence or other adhesion molecule binding site). Within one such embodiment, the cyclic peptide contains 2 HAV sequences. Within another embodiment, the cyclic peptide contains one HAV and one CAR sequence recognized by a different CAM. In a preferred embodiment, the second CAR sequence is derived from fibronectin and is recognized by an integrin (*i.e.*, Arg-Gly-Asp; *see* Cardarelli et al., *J. Biol. Chem. 267*:23159-23164, 1992).

In addition to the CAR sequence(s), cyclic peptides generally comprise at least one additional residue, such that the size of the cyclic peptide ring ranges from 4 to about 15 residues, preferably from 5 to 10 residues. Such additional residue(s) may be present on the N-terminal and/or C-terminal side of a CAR sequence, and may be derived from sequences that flank the HAV sequence within one or more naturally occurring cadherins (*e.g.*, N-cadherin, E-cadherin, P-cadherin, R-cadherin or other cadherins containing the HAV sequence) with or without amino acid substitutions and/or other modifications. Flanking sequences for endogenous N-, E-, P- and R-cadherin are shown in Figure 2, and in SEQ ID NOs: 1 to 7. Database accession numbers for representative naturally occurring cadherins are as follows: human N-cadherin M34064, mouse N-cadherin M31131 and M22556, cow N-cadherin X53615, human P-cadherin X63629, mouse P-cadherin X06340, human E-cadherin Z13009, mouse E-cadherin X06115. Alternatively, additional residues present on one or both sides of the CAR sequence(s) may be unrelated to an endogenous sequence (*e.g.*, residues that facilitate cyclization).

Within certain preferred embodiments, as discussed below, relatively small cyclic peptides that do not contain significant sequences flanking the HAV sequence are preferred for modulating N-cadherin and E-cadherin mediated cell adhesion. Such peptides may contain an N-acetyl group and a C-amide group (*e.g.*, the 5-residue ring N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8) or N-Ac-KHAVD-NH$_2$ (SEQ ID NO:20)). The finding, within the present invention, that such relatively small cyclic peptides may be effective and all-purpose inhibitors of cell adhesion represents a unexpected discovery. Such cyclic peptides can be thought of as "master keys" that fit into peptide binding sites of each of the different classical cadherins, and are capable of

disrupting cell adhesion of neural cells, endothelial cells, epithelial cells and/or certain cancer cells. Small cyclic peptides may generally be used to specifically modulate cell adhesion of neural and/or other cell types by topical administration or by systemic administration, with or without linking a targeting agent to the peptide, as discussed

5   below.

Within other preferred embodiments, a cyclic peptide may contain sequences that flank the HAV sequence on one or both sides that are designed to confer specificity for cell adhesion mediated by one or more specific cadherins, resulting in tissue and/or cell-type specificity. Suitable flanking sequences for conferring specificity

10   include, but are not limited to, endogenous sequences present in one or more naturally occurring cadherins, and cyclic peptides having specificity may be identified using the representative screens provided herein. For example, it has been found, within the context of the present invention, that cyclic peptides that contain additional residues derived from the native E-cadherin sequence on the C-terminal side of the CAR

15   sequence are specific for epithelial cells (*i.e.*, such peptides disrupt E-cadherin mediated cell adhesion to a greater extent than they disrupt N-cadherin expression). The addition of appropriate endogenous sequences may similarly result in peptides that disrupt N-cadherin mediated cell adhesion.

To facilitate the preparation of cyclic peptides having a desired

20   specificity, nuclear magnetic resonance (NMR) and computational techniques may be used to determine the conformation of a peptide that confers a known specificity. NMR is widely used for structural analysis of molecules. Cross-peak intensities in nuclear Overhauser enhancement (NOE) spectra, coupling constants and chemical shifts depend on the conformation of a compound. NOE data provide the interproton distance

25   between protons through space and across the ring of the cyclic peptide. This information may be used to facilitate calculation of the lowest energy conformation for the HAV sequence. Conformation may then be correlated with tissue specificity to permit the identification of peptides that are similarly tissue specific or have enhanced tissue specificity.

30   Cyclic peptides as described herein may comprise residues of L-amino acids, D-amino acids, or any combination thereof. Amino acids may be from natural or

non-natural sources, provided that at least one amino group and at least one carboxyl group are present in the molecule; α- and β-amino acids are generally preferred. The 20 L-amino acids commonly found in proteins are identified herein by the conventional three-letter or one-letter abbreviations indicated in Table 1, and the corresponding D-
5 amino acids are designated by a lower case one letter symbol. A cyclic peptide may also contain one or more rare amino acids (such as 4-hydroxyproline or hydroxylysine), organic acids or amides and/or derivatives of common amino acids, such as amino acids having the C-terminal carboxylate esterified (*e.g.*, benzyl, methyl or ethyl ester) or amidated and/or having modifications of the N-terminal amino group (*e.g.*, acetylation
10 or alkoxycarbonylation), with or without any of a wide variety of side-chain modifications and/or substitutions (*e.g.*, methylation, benzylation, t-butylation, tosylation, alkoxycarbonylation, and the like). Preferred derivatives include amino acids having an N-acetyl group (such that the amino group that represents the N-terminus of the linear peptide prior to cyclization is acetylated) and/or a C-terminal
15 amide group (*i.e.*, the carboxy terminus of the linear peptide prior to cyclization is amidated). Residues other than common amino acids that may be present with a cyclic peptide include, but are not limited to, penicillamine, β,β-tetramethylene cysteine, β,β-pentamethylene cysteine, β-mercaptopropionic acid, β,β-pentamethylene-β-mercaptopropionic acid, 2-mercaptobenzene, 2-mercaptoaniline, 2-mercaptoproline,
20 ornithine, diaminobutyric acid, α-aminoadipic acid, m-aminomethylbenzoic acid and α,β-diaminopropionic acid.

Table 1

Amino acid one-letter and three-letter abbreviations

A	Ala	Alanine
R	Arg	Arginine
D	Asp	Aspartic acid
N	Asn	Asparagine
C	Cys	Cysteine
Q	Gln	Glutamine
E	Glu	Glutamic acid
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
L	Leu	Leucine
K	Lys	Lysine
M	Met	Methionine
F	Phe	Phenylalanine
P	Pro	Proline
S	Ser	Serine
T	Thr	Threonine
W	Trp	Tryptophan
Y	Tyr	Tyrosine
V	Val	Valine

Cyclic peptides as described herein may be synthesized by methods well known in the art, including recombinant DNA methods and chemical synthesis. Chemical synthesis may generally be performed using standard solution phase or solid phase peptide synthesis techniques, in which a peptide linkage occurs through the direct condensation of the α-amino group of one amino acid with the α-carboxy group of the other amino acid with the elimination of a water molecule. Peptide bond synthesis by direct condensation, as formulated above, requires suppression of the reactive character of the amino group of the first and of the carboxyl group of the second amino acid. The masking substituents must permit their ready removal, without inducing breakdown of the labile peptide molecule.

In solution phase synthesis, a wide variety of coupling methods and protecting groups may be used (see Gross and Meienhofer, eds., "The Peptides: Analysis, Synthesis, Biology," Vol. 1-4 (Academic Press, 1979); Bodansky and Bodansky, "The Practice of Peptide Synthesis," 2d ed. (Springer Verlag, 1994)). In addition, intermediate purification and linear scale up are possible. Those of ordinary skill in the art will appreciate that solution synthesis requires consideration of main chain and side chain protecting groups and activation method. In addition, careful segment selection is necessary to minimize racemization during segment condensation. Solubility considerations are also a factor.

Solid phase peptide synthesis uses an insoluble polymer for support during organic synthesis. The polymer-supported peptide chain permits the use of simple washing and filtration steps instead of laborious purifications at intermediate steps. Solid-phase peptide synthesis may generally be performed according to the method of Merrifield et al., J. Am. Chem. Soc. 85:2149, 1963, which involves assembling a linear peptide chain on a resin support using protected amino acids. Solid phase peptide synthesis typically utilizes either the Boc or Fmoc strategy. The Boc strategy uses a 1% cross-linked polystyrene resin. The standard protecting group for α-amino functions is the tert-butyloxycarbonyl (Boc) group. This group can be removed with dilute solutions of strong acids such as 25% trifluoroacetic acid (TFA). The next Boc-amino acid is typically coupled to the amino acyl resin using dicyclohexylcarbodiimide (DCC). Following completion of the assembly, the peptide-resin is treated with anhydrous HF to cleave the benzyl ester link and liberate the free peptide. Side-chain functional groups are usually blocked during synthesis by benzyl-derived blocking groups, which are also cleaved by HF. The free peptide is then extracted from the resin with a suitable solvent, purified and characterized. Newly synthesized peptides can be purified, for example, by gel filtration, HPLC, partition chromatography and/or ion-exchange chromatography, and may be characterized by, for example, mass spectrometry or amino acid sequence analysis. In the Boc strategy, C-terminal amidated peptides can be obtained using benzhydrylamine or methylbenzhydrylamine resins, which yield peptide amides directly upon cleavage with HF.
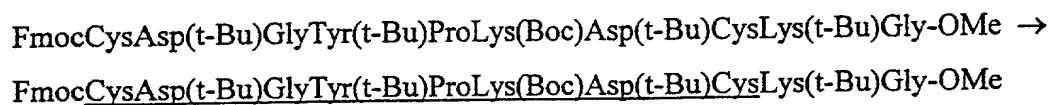
In the procedures discussed above, the selectivity of the side-chain blocking groups and of the peptide-resin link depends upon the differences in the rate of acidolytic cleavage. Orthoganol systems have been introduced in which the side-chain blocking groups and the peptide-resin link are completely stable to the reagent used to remove the α-protecting group at each step of the synthesis. The most common of these methods involves the 9-fluorenylmethyloxycarbonyl (Fmoc) approach. Within this method, the side-chain protecting groups and the peptide-resin link are completely stable to the secondary amines used for cleaving the N-α-Fmoc group. The side-chain protection and the peptide-resin link are cleaved by mild acidolysis. The repeated contact with base makes the Merrifield resin unsuitable for Fmoc chemistry, and p-alkoxybenzyl esters linked to the resin are generally used. Deprotection and cleavage are generally accomplished using TFA.

Those of ordinary skill in the art will recognize that, in solid phase synthesis, deprotection and coupling reactions must go to completion and the side-chain blocking groups must be stable throughout the entire synthesis. In addition, solid phase synthesis is generally most suitable when peptides are to be made on a small scale.
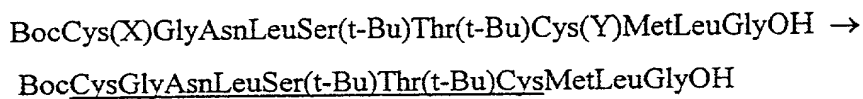
Acetylation of the N-terminal can be accomplished by reacting the final peptide with acetic anhydride before cleavage from the resin. C-amidation is accomplished using an appropriate resin such as methylbenzhydrylamine resin using the Boc technology.

Following synthesis of a linear peptide, with or without N-acetylation and/or C-amidation, cyclization may be achieved by any of a variety of techniques well known in the art. Within one embodiment, a bond may be generated between reactive amino acid side chains. For example, a disulfide bridge may be formed from a linear peptide comprising two thiol-containing residues by oxidizing the peptide using any of a variety of methods. Within one such method, air oxidation of thiols can generate disulfide linkages over a period of several days using either basic or neutral aqueous media. The peptide is used in high dilution to minimize aggregation and intermolecular side reactions. This method suffers from the disadvantage of being slow but has the advantage of only producing H_2O as a side product. Alternatively, strong oxidizing agents such as I_2 and $K_3Fe(CN)_6$ can be used to form disulfide linkages. Those of

ordinary skill in the art will recognize that care must be taken not to oxidize the sensitive side chains of Met, Tyr, Trp or His. Cyclic peptides produced by this method require purification using standard techniques, but this oxidation is applicable at acid pHs. By way of example, strong oxidizing agents can be used to perform the

5   cyclization shown below (SEQ ID NOs: 33 and 34), in which the underlined portion is cyclized:

FmocCysAsp(t-Bu)GlyTyr(t-Bu)ProLys(Boc)Asp(t-Bu)CysLys(t-Bu)Gly-OMe →

FmocCysAsp(t-Bu)GlyTyr(t-Bu)ProLys(Boc)Asp(t-Bu)CysLys(t-Bu)Gly-OMe

10

Oxidizing agents also allow concurrent deprotection/oxidation of suitable S-protected linear precursors to avoid premature, nonspecific oxidation of free cysteine, as shown below (SEQ ID Nos: 35 and 36), where X and Y = S-Trt or S-Acm:

15   BocCys(X)GlyAsnLeuSer(t-Bu)Thr(t-Bu)Cys(Y)MetLeuGlyOH →

BocCysGlyAsnLeuSer(t-Bu)Thr(t-Bu)CysMetLeuGlyOH

DMSO, unlike I_2 and $K_3Fe(CN)_6$, is a mild oxidizing agent which does not cause oxidative side reactions of the nucleophilic amino acids mentioned above.

20   DMSO is miscible with H_2O at all concentrations, and oxidations can be performed at acidic to neutral pHs with harmless byproducts. Methyltrichlorosilane-diphenylsulfoxide may alternatively be used as an oxidizing agent, for concurrent deprotection/oxidation of S-Acm, S-Tacm or S-t-Bu of cysteine without affecting other nucleophilic amino acids. There are no polymeric products resulting from

25   intermolecular disulfide bond formation. In the example below (SEQ ID NOs: 37 and 38), X is Acm, Tacm or t-Bu:

H-Cys(X)TyrIleGlnAsnCys(X)ProLeuGly-NH₂ →

H-CysTyrIleGlnAsnCysProLeuGly-NH₂

30
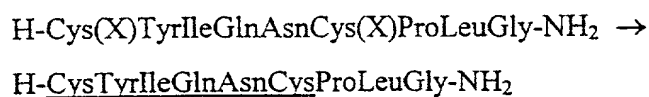
Suitable thiol-containing residues for use in such oxidation methods include, but are not limited to, cysteine, β,β-dimethyl cysteine (penicillamine or Pen), β,β-tetramethylene cysteine (Tmc), β,β-pentamethylene cysteine (Pmc), β-mercaptopropionic acid (Mpr), β,β-pentamethylene-β-mercaptopropionic acid (Pmp), 2-mercaptobenzene, 2-mercaptoaniline and 2-mercaptoproline. Peptides containing such residues are illustrated by the following representative formulas, in which the underlined portion is cyclized, N-acetyl groups are indicated by N-Ac and C-terminal amide groups are represented by -NH$_2$:

i)   N-Ac-Cys-His-Ala-Val-Cys-NH$_2$ (SEQ ID NO:8)

ii)   N-Ac-Cys-Ala-His-Ala-Val-Asp-Ile-Cys-NH$_2$ (SEQ ID NO:10)

iii) N-Ac-Cys-Ser-His-Ala-Val-Cys-NH$_2$ (SEQ ID NO:12)

iv) N-Ac-Cys-His-Ala-Val-Ser-Cys-NH$_2$ (SEQ ID NO:14)

v)   N-Ac-Cys-Ala-His-Ala-Val-Asp-Cys-NH$_2$ (SEQ ID NO:16)

vi) N-Ac-Cys-Ser-His-Ala-Val-Ser-Ser-Cys-NH$_2$ (SEQ ID NO:18)

vii) N-Ac-Cys-His-Ala-Val-Ser-Cys-OH (SEQ ID NO:14)

viii) H-Cys-Ala-His-Ala-Val-Asp-Cys-NH$_2$ (SEQ ID NO:16)

ix) N-Ac-Cys-His-Ala-Val-Pen-NH$_2$ (SEQ ID NO:28)

x)   N-Ac-Ile-Tmc-Tyr-Ser-His-Ala-Val-Ser-Cys-Glu-NH$_2$ (SEQ ID NO:29)

xi) N-Ac-Ile-Pmc-Tyr-Ser-His-Ala-Val-Ser-Ser-Cys-NH$_2$ (SEQ ID NO:30)

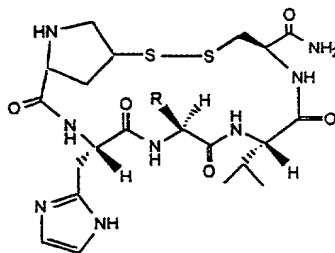xii)   Mpr-Tyr-Ser-His-Ala-Val-Ser-Ser-Cys-NH₂ (SEQ ID NO:31)

xiii)   Pmp-Tyr-Ser-His-Ala-Val-Ser-Ser-Cys-NH₂ (SEQ ID NO:32)

5   xii)
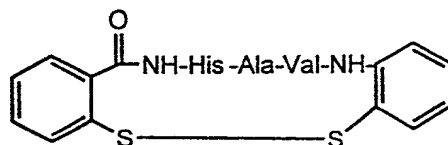
xiii)

10

It will be readily apparent to those of ordinary skill in the art that, within each of these representative formulas, any of the above thiol-containing residues may be 15 employed in place of one or both of the thiol-containing residues recited.

Within another embodiment, cyclization may be achieved by amide bond formation. For example, a peptide bond may be formed between terminal functional groups (*i.e.*, the amino and carboxy termini of a linear peptide prior to cyclization). Two such cyclic peptides are AHAVDI (SEQ ID NO:44) and SHAVSS (SEQ ID 20 NO:45), with or without an N-terminal acetyl group and/or a C-terminal amide. Within another such embodiment, the linear peptide comprises a D-amino acid (*e.g.*, HAVsS). Alternatively, cyclization may be accomplished by linking one terminus and a residue side chain or using two side chains, as in KHAVD (SEQ ID NO:20) or KSHAVSSD (SEQ ID NO:46), with or without an N-terminal acetyl group and/or a C-terminal 25 amide. Residues capable of forming a lactam bond include lysine, ornithine (Orn), α-

amino adipic acid, m-aminomethylbenzoic acid, α,β-diaminopropionic acid, glutamate or aspartate.

Methods for forming amide bonds are well known in the art and are based on well established principles of chemical reactivity. Within one such method, carbodiimide-mediated lactam formation can be accomplished by reaction of the carboxylic acid with DCC, DIC, EDAC or DCCI, resulting in the formation of an O-acylurea that can be reacted immediately with the free amino group to complete the cyclization. The formation of the inactive N-acylurea, resulting from O→N migration, can be circumvented by converting the O-acylurea to an active ester by reaction with an N-hydroxy compound such as 1-hydroxybenzotriazole, 1-hydroxysuccinimide, 1-hydroxynorbornene carboxamide or ethyl 2-hydroximino-2-cyanoacetate. In addition to minimizing O→N migration, these additives also serve as catalysts during cyclization and assist in lowering racemization. Alternatively, cyclization can be performed using the azide method, in which a reactive azide intermediate is generated from an alkyl ester via a hydrazide. Hydrazinolysis of the terminal ester necessitates the use of a t-butyl group for the protection of side chain carboxyl functions in the acylating component. This limitation can be overcome by using diphenylphosphoryl acid (DPPA), which furnishes an azide directly upon reaction with a carboxyl group. The slow reactivity of azides and the formation of isocyanates by their disproportionation restrict the usefulness of this method. The mixed anhydride method of lactam formation is widely used because of the facile removal of reaction by-products. The anhydride is formed upon reaction of the carboxylate anion with an alkyl chloroformate or pivaloyl chloride. The attack of the amino component is then guided to the carbonyl carbon of the acylating component by the electron donating effect of the alkoxy group or by the steric bulk of the pivaloyl chloride t-butyl group, which obstructs attack on the wrong carbonyl group. Mixed anhydrides with phosphoric acid derivatives have also been successfully used. Alternatively, cyclization can be accomplished using activated esters. The presence of electron withdrawing substituents on the alkoxy carbon of esters increases their susceptibility to aminolysis. The high reactivity of esters of p-nitrophenol, N-hydroxy compounds and polyhalogenated phenols has made these "active esters" useful in the synthesis of amide bonds. The last few years have

witnessed the development of benzotriazolyloxytris-(dimethylamino)phosphonium hexafluorophosphonate (BOP) and its congeners as advantageous coupling reagents. Their performance is generally superior to that of the well established carbodiimide amide bond formation reactions.

5   Within a further embodiment, a thioether linkage may be formed between the side chain of a thiol-containing residue and an appropriately derivatized α-amino acid. By way of example, a lysine side chain can be coupled to bromoacetic acid through the carbodiimide coupling method (DCC, EDAC) and then reacted with the side chain of any of the thiol containing residues mentioned above to form a thioether

10   linkage. In order to form dithioethers, any two thiol containing side-chains can be reacted with dibromoethane and diisopropylamine in DMF. Examples of thiol-containing linkages are shown below:

i.

$X = (CH_2)_4$

$= CH_2$

$=$ <img>phenyl</img>$-CH_2$

ii.
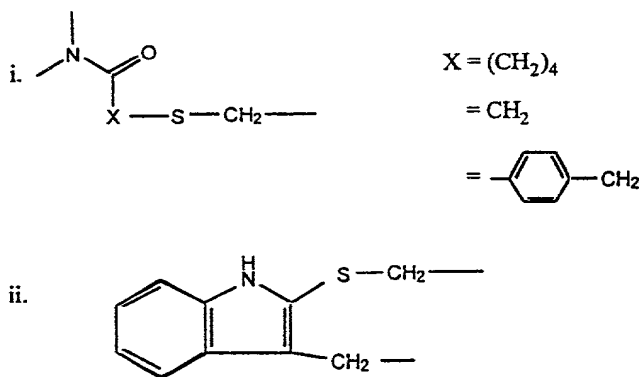
15

Cyclization may also be achieved using δ_1,δ_1-Ditryptophan (*i.e.*, Ac-Trp-Gly-Gly-Trp-OMe) (SEQ ID NO:39), as shown below:
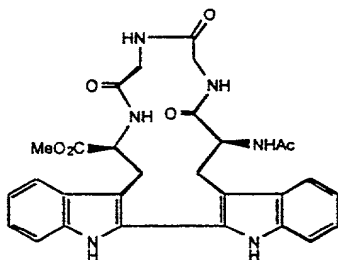
20

Representative structures of cyclic peptides are provided in Figure 3.
Within Figure 3, certain cyclic peptides having the ability to modulate cell adhesion (shown on the left) are paired with similar inactive structures (on the right). The structures and formulas recited herein are provided solely for the purpose of illustration, and are not intended to limit the scope of the cyclic peptides described herein.

EVALUATION OF CYCLIC PEPTIDE ACTIVITY

As noted above, cyclic peptides as described herein are capable of modulating (i.e., enhancing or inhibiting) cadherin-mediated cell adhesion. The ability of a peptide to modulate cell adhesion may generally be evaluated in vitro by assaying the effect of the cyclic peptide on one or more of the following: (1) neurite outgrowth, (2) adhesion between endothelial cells, (3) adhesion between epithelial cells (e.g., normal rat kidney cells and/or human skin) and/or (4) adhesion between cancer cells. For the purpose of such assays, peptides that are not linked to a support material or other compound (as described below) are generally evaluated. In general, a cyclic peptide is considered to be a modulator of cell adhesion if, within one or more of these representative assays, contact of the test cells with the peptide results in a discernible disruption of cell adhesion.

Within a representative neurite outgrowth assay, neurons may be cultured on a monolayer of cells (e.g., 3T3) that express N-cadherin. Neurons grown on such cells (under suitable conditions and for a sufficient period of time) extend longer neurites than neurons cultured on cells that do not express N-cadherin. A cyclic peptide that modulates cadherin-mediated cell adhesion may inhibit such neurite outgrowth. Suitable growth conditions for cerebellar neurons include growth for about 18 hours in SATO/2%FCS medium. Under such conditions, the presence of 500 μg/mL cyclic peptide is sufficient to disrupt neural cell adhesion, as measured by a decrease in the mean neurite length of at least 50%, relative to the length in the absence of cyclic peptide or in the presence of a negative control peptide.

Within one representative cell adhesion assay, the addition of a cyclic peptide to cells that express a cadherin results in disruption of cell adhesion. A "cadherin-expressing cell," as used herein, may be any type of cell that expresses at least one cadherin on the cell surface at a detectable level, using standard techniques such as

5   immunocytochemical protocols (Blaschuk and Farookhim, *Dev. Biol.* *136*:564-567, 1989). Cadherin-expressing cells include endothelial (*e.g.*, bovine pulmonary artery endothelial cells), epithelial and/or cancer cells (*e.g.*, the human ovarian cancer cell line SKOV3 (ATCC #HTB-77)). For example, such cells may be plated under standard conditions that permit cell adhesion in the presence and absence of cyclic peptide (*e.g.*,

10   500 μg/mL). Disruption of cell adhesion may be determined visually within 24 hours, by observing retraction of the cells from one another.

Within another such assay, the effect of a cyclic peptide on normal rat kidney (NRK-52E; ATCC #1571-CRL) cells may be evaluated. In the absence of cyclic peptide, NRK cells form characteristic tightly adherent monolayers with a cobblestone

15   morphology in which cells display a polygonal shape. NRK cells that are treated with a cyclic peptide typically assume a non-polygonal and elongated morphology (*i.e.*, a fibroblast-like shape) within 48 hours of treatment with 1 mg/mL of peptide. Gaps appear in confluent cultures of such cells. In addition, 1 mg/mL of such a peptide reproducibly induces a readily apparent reduction in cell surface staining of E-cadherin,

20   as judged by immunofluorescence microscopy (Laird et al., *J. Cell Biol.* *131*:1193-1203, 1995), of at least 75% within 48 hours.

A third cell adhesion assay involves evaluating the effect of a cyclic peptide on permeability of adherent epithelial and/or endothelial cell layers. For example, the effect of permeability on human skin may be evaluated. Such skin may be

25   derived from a natural source or may be synthetic. Human abdominal skin for use in such assays may generally be obtained from humans at autopsy within 24 hours of death. Briefly, a cyclic peptide and a test marker (*e.g.*, the fluorescent markers Oregon Green™ and Rhodamine Green™ Dextran) may be dissolved in a sterile buffer, and the ability of the marker to penetrate through the skin and into a receptor fluid may be

30   measured using a Franz Cell apparatus (Franz, *Curr. Prob. Dermatol.* *7*:58-68, 1978; Franz, *J. Invest. Dermatol.* *64*:190-195, 1975). In general, a cyclic peptide that

enhances the permeability of human skin results in a statistically significant increase in the amount of marker in the receptor compartment after 6-48 hours in the presence of 500 μg/mL peptide.

5 CYCLIC PEPTIDE MODIFICATION AND FORMULATIONS

A cyclic peptide as described herein may, but need not, be linked to one or more additional molecules. For example, two or more cyclic peptides may be linked together, using well known techniques as discussed below. Within certain embodiments, the linked cyclic peptides may contain HAV sequences and may be

10 joined by a linker, which may be a peptide and/or non-peptide sequence. Preferably, the linker generates a separation distance between recognition sites of 50-200μ. One linker that can be used for such purposes is $H_2N(CH_2)_nCO_2H$, or derivatives thereof, where n ranges from 1-10. Other linkers that may be used will be apparent to those of ordinary skill in the art. Cyclic peptides linked in this manner may generally be used within

15 methods in which enhancement of cadherin-mediated cell adhesion is desired.

Alternatively, a cyclic peptide as described herein may be linked to a molecule comprising a cell adhesion recognition sequence for a different adhesion molecule (including, but not limited to, other CAMs), preferably separated by a linker. As used herein, an "adhesion molecule" is any molecule that mediates cell adhesion via

20 a receptor on the cell's surface. Adhesion molecules include cell adhesion proteins (such as integrins and members of the immunoglobulin gene superfamily such as N-CAM, as well as extracellular matrix protein) such as laminin, fibronectin, collagens, vitronectin and tenascin. Preferred cell adhesion recognition sequences for linking to a cyclic peptide include Arg-Gly-Asp, which is bound by integrins, and Tyr-Ile-Gly-Ser-

25 Arg (SEQ ID NO:47), which is bound by laminins. Cyclic peptides that are linked to such molecules (which may themselves be cyclic) may be used within methods in which it is desirable to disrupt cell adhesion mediated by multiple adhesion molecules.

In addition, as discussed below, it may be beneficial for certain applications to link one or more cyclic peptides to a gel or solid support material, such

30 as a polymeric matrix (which may be formulated as a membrane or microstructure, such as an ultra thin film), a container surface (e.g., the surface of a tissue culture plate or the

interior surface of a bioreactor), or a bead or other particle, which may be prepared from a variety of materials including glass, plastic or ceramics. For certain applications, biodegradable support materials are preferred, such as cellulose and derivatives thereof, collagen or any of a variety of polyesters (*e.g.,* those derived from hydroxy acids and/or lactones), spider silk or sutures (*see* U.S. Patent No. 5,245,012). Suitable methods for linking a cyclic peptide to a support material will depend upon the composition of the support and the intended use, and will be readily apparent to those of ordinary skill in the art. Attachment may generally be achieved through noncovalent association, such as adsorption or affinity or, preferably, via covalent attachment (which may be a direct linkage between a cyclic peptide and functional groups on the support, or may be a linkage by way of a cross-linking agent). Attachment of a cyclic peptide by adsorption may be achieved by contact, in a suitable buffer, with a solid support for a suitable amount of time. The contact time varies with temperature, but is generally between about 5 seconds and 1 day, and typically between about 10 seconds and 1 hour.

Covalent attachment of a cyclic peptide to a solid support may generally be achieved by first reacting the support with a linker or bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the cyclic peptide. For example, a cyclic peptide may be bound to an appropriate polymeric support or coating using benzoquinone, by condensation of an aldehyde group on the support with an amine and an active hydrogen on the cyclic peptide or by condensation of an amino group on the support with a carboxylic acid on the cyclic peptide. A preferred method of generating a linkage is via amino groups using glutaraldehyde. A cyclic peptide may be linked to cellulose via ester linkages. Similarly, amide linkages may be suitable for other support materials such as keyhole limpet hemocyanin.

Although cyclic peptides as described herein may preferentially bind to specific tissues or cells, and thus may be sufficient to target a desired site *in vivo*, it may be beneficial for certain applications to include an additional targeting agent. Accordingly, a targeting agent may also, or alternatively, be linked to a cyclic peptide to facilitate targeting to one or more specific tissues. As used herein, a "targeting agent," may be any substance (such as a compound or cell) that, when linked to a peptide

enhances the transport of the peptide to a target tissue, thereby increasing the local concentration of the cyclic peptide. Targeting agents include antibodies or fragments thereof, receptors, ligands and other molecules that bind to cells of, or in the vicinity of, the target tissue. Known targeting agents include hormones, antibodies against cell

5   surface antigens, lectins, adhesion molecules, tumor cell surface binding ligands, steroids, cholesterol, lymphokines, fibrinolytic enzymes and other drugs and proteins that bind to a desired target site. Among the many monoclonal antibodies that may serve as targeting agents are anti-TAC, or other interleukin-2 receptor antibodies; 9.2.27 and NR-ML-05, reactive with the 250 kilodalton human melanoma-associated

10   proteoglycan; and NR-LU-10, reactive with a pancarcinoma glycoprotein. The antibody employed in the present invention may be an intact (whole) molecule, a fragment thereof, or a functional equivalent thereof. Examples of antibody fragments are F(ab')2, -Fab', Fab and F[v] fragments, which may be produced by conventional methods or by genetic or protein engineering. Linkage may be via any suitable covalent bond using

15   standard techniques that are well known in the art. Such linkage is generally covalent and may be achieved by, for example, direct condensation or other reactions, or by way of bi- or multi-functional linkers.

        For certain embodiments, it may be beneficial to also, or alternatively, link a drug to a cyclic peptide. As used herein, the term "drug" refers to any bioactive

20   agent intended for administration to a mammal to prevent or treat a disease or other undesirable condition. Drugs include hormones, growth factors, proteins, peptides and other compounds. The use of certain specific drugs within the context of the present invention is discussed below.

        Within certain aspects of the present invention, one or more cyclic

25   peptides as described herein may be present within a pharmaceutical composition. A pharmaceutical composition comprises one or more cyclic peptides in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers (*e.g.*, neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans),

30   mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione, adjuvants (*e.g.*, aluminum hydroxide) and/or

preservatives. Within yet other embodiments, compositions of the present invention may be formulated as a lyophilizate. A cyclic peptide (alone or in combination with a targeting agent and/or drug) may, but need not, be encapsulated within liposomes using well known technology. Compositions of the present invention may be formulated for

5 any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous, or intramuscular administration. For certain topical applications, formulation as a cream or lotion, using well known components, is preferred.

A pharmaceutical composition may also contain one or more drugs,

10 which may be linked to a cyclic peptide or may be free within the composition. Virtually any drug may be administered in combination with a cyclic peptide as described herein, for a variety of purposes as described below. Examples of types of drugs that may be administered with a cyclic peptide include analgesics, anesthetics, antianginals, antifungals, antibiotics, anticancer drugs (e.g., taxol or mitomycin C),

15 antiinflammatories (e.g., ibuprofin and indomethacin), anthelmintics, antidepressants, antidotes, antiemetics, antihistamines, antihypertensives, antimalarials, antimicrotubule agents (e.g., colchicine or vinca alkaloids), antimigraine agents, antimicrobials, antiphsychotics, antipyretics, antiseptics, anti-signaling agents (e.g., protein kinase C inhibitors or inhibitors of intracellular calcium mobilization), antiarthritics,

20 antithrombin agents, antituberculotics, antitussives, antivirals, appetite suppresants, cardioactive drugs, chemical dependency drugs, cathartics, chemotherapeutic agents, coronary, cerebral or peripheral vasodilators, contraceptive agents, depressants, diuretics, expectorants, growth factors, hormonal agents, hypnotics, immunosuppression agents, narcotic antagonists, parasympathomimetics, sedatives, stimulants,

25 sympathomimetics, toxins (e.g., cholera toxin), tranquilizers and urinary antiinfectives.

For imaging purposes, any of a variety of diagnostic agents may be incorporated into a pharmaceutical composition, either linked to a cyclic peptide or free within the composition. Diagnostic agents include any substance administered to illuminate a physiological function within a patient, while leaving other physiological

30 functions generally unaffected. Diagnostic agents include metals, radioactive isotopes and radioopaque agents (e.g., gallium, technetium, indium, strontium, iodine, barium,

bromine and phosphorus-containing compounds), radiolucent agents, contrast agents, dyes (*e.g.,* fluorescent dyes and chromophores) and enzymes that catalyze a colorimetric or fluorometric reaction. In general, such agents may be attached using a variety of techniques as described above, and may be present in any orientation.

5   The compositions described herein may be administered as part of a sustained release formulation (*i.e.,* a formulation such as a capsule or sponge that effects a slow release of cyclic peptide following administration). Such formulations may generally be prepared using well known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. 10 Sustained-release formulations may contain a cyclic peptide dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane (*see, e.g.,* European Patent Application 710,491 A). Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of cyclic peptide release. The amount 15 of cyclic peptide contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

    Pharmaceutical compositions of the present invention may be administered in a manner appropriate to the disease to be treated (or prevented). 20 Appropriate dosages and the duration and frequency of administration will be determined by such factors as the condition of the patient, the type and severity of the patient's disease and the method of administration. In general, an appropriate dosage and treatment regimen provides the cyclic peptide(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Within particularly preferred embodiments of 25 the invention, a cyclic peptide or pharmaceutical composition as described herein may be administered at a dosage ranging from 0.001 to 50 mg/kg body weight, preferably from 0.1 to 20 mg/kg, on a regimen of single or multiple daily doses. For topical administration, a cream typically comprises an amount of cyclic peptide ranging from 0.00001% to 1%, preferably 0.0001% to 0.002%. Fluid compositions typically contain 30 about 10 ng/ml to 5 mg/ml, preferably from about 10 µg to 2 mg/mL cyclic peptide. Appropriate dosages may generally be determined using experimental models and/or

clinical trials. In general, the use of the minimum dosage that is sufficient to provide effective therapy is preferred. Patients may generally be monitored for therapeutic effectiveness using assays suitable for the condition being treated or prevented, which will be familiar to those of ordinary skill in the art.

CYCLIC PEPTIDE METHODS OF USE

In general, the cyclic peptides and compositions described herein may be used for modulating the adhesion of cadherin-expressing cells (*i.e.*, cells that express one or more of E-cadherin, N-cadherin, P-cadherin, R-cadherin and/or other cadherin(s) containing the HAV sequence, including as yet undiscovered cadherins) *in vitro* and/or *in vivo*. In general, the methods described herein have an advantage over prior techniques in that they permit the passage of molecules that are large and/or charged across barriers of cadherin-expressing cells. As discussed in greater detail below, cyclic peptides as described herein may also be used to disrupt or enhance cell adhesion in a variety of other contexts. Within the methods described herein, one or more cyclic peptides may generally be administered alone, or within a pharmaceutical composition. In each specific method described herein, as noted above, a targeting agent may be employed to increase the local concentration of cyclic peptide at the target site.

In one such aspect, the present invention provides methods for reducing unwanted cellular adhesion by administering a cyclic peptide as described herein. Unwanted cellular adhesion can occur between tumor cells, between tumor cells and normal cells or between normal cells as a result of surgery, injury, chemotherapy, disease, inflammation or other condition jeopardizing cell viability or function. Preferred cyclic peptides for use within such methods include N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8), N-Ac-CHAVSC-NH$_2$ (SEQ ID NO:14), N-Ac-CAHAVDC-NH$_2$ (SEQ ID NO:16), N-Ac-CSHAVSSC-NH$_2$ (SEQ ID NO:18) and N-Ac-KHAVD-NH$_2$ (SEQ ID NO:20). Topical administration of the cyclic peptide(s) is generally preferred, but other means may also be employed. Preferably, a fluid composition for topical administration (comprising, for example, physiological saline) comprises an amount of cyclic peptide as described above, and more preferably an amount ranging from 10μg/mL to 1mg/mL. Creams may generally be formulated as described above.

Topical administration in the surgical field may be given once at the end of surgery by irrigation of the wound, as an intermittent or continuous irrigation with use of surgical drains in the post operative period, or by the use of drains specifically inserted in an area of inflammation, injury or disease in cases where surgery does not need to be

5   performed. Alternatively, parenteral or transcutaneous administration may be used to achieve similar results.

In another aspect, methods are provided for enhancing the delivery of a drug through the skin of a mammal. Transdermal delivery of drugs is a convenient and non-invasive method that can be used to maintain relatively constant blood levels of a

10   drug. In general, to facilitate drug delivery via the skin, it is necessary to perturb adhesion between the epithelial cells (keratinocytes) and the endothelial cells of the microvasculature. Using currently available techniques, only small, uncharged molecules may be delivered across skin *in vivo*. The methods described herein are not subject to the same degree of limitation. Accordingly, a wide variety of drugs may be

15   transported across the epithelial and endothelial cell layers of skin, for systemic or topical administration. Such drugs may be delivered to melanomas or may enter the blood stream of the mammal for delivery to other sites within the body.

To enhance the delivery of a drug through the skin, a cyclic peptide as described herein and a drug are contacted with the skin surface. Preferred cyclic

20   peptides for use within such methods comprise an N-acetyl group, as described above, such as N-Ac-$\underline{CHAVC}$-NH$_2$ (SEQ ID NO:8). Contact may be achieved by direct application of the cyclic peptide, generally within a composition formulated as a cream or gel, or using any of a variety of skin contact devices for transdermal application (such as those described in European Patent Application No. 566,816 A; U.S. Patent No.

25   5,613,958; U.S. Patent No. 5,505,956). A skin patch provides a convenient method of administration (particularly for slow-release formulations). Such patches may contain a reservoir of cyclic peptide and drug separated from the skin by a membrane through which the drug diffuses. Within other patch designs, the cyclic peptide and drug may be dissolved or suspended in a polymer or adhesive matrix that is then placed in direct

30   contact with the patient's skin. The cyclic peptide and drug may then diffuse from the matrix into the skin. Cyclic peptide(s) and drug(s) may be contained within the same

composition or skin patch, or may be separately administered, although administration at the same time and site is preferred. In general, the amount of cyclic peptide administered via the skin varies with the nature of the condition to be treated or prevented, but may vary as described above. Such levels may be achieved by

5 appropriate adjustments to the device used, or by applying a cream formulated as described above. Transfer of the drug across the skin and to the target tissue may be predicted based on *in vitro* studies using, for example, a Franz cell apparatus, and evaluated *in vivo* by appropriate means that will be apparent to those of ordinary skill in the art. As an example, monitoring of the serum level of the administered drug over

10 time provides a convenient measure of the drug transfer across the skin.

Transdermal drug delivery as described herein is particularly useful in situations in which a constant rate of drug delivery is desired, to avoid fluctuating blood levels of a drug. For example, morphine is an analgesic commonly used immediately following surgery. When given intermittently in a parenteral form (intramuscular,

15 intravenous), the patient usually feels sleepy during the first hour, is well during the next 2 hours and is in pain during the last hour because the blood level goes up quickly after the injection and goes down below the desirable level before the 4 hour interval prescribed for re-injection is reached. Transdermal administration as described herein permits the maintenance of constant levels for long periods of time (*e.g.*, days), which

20 allows adequate pain control and mental alertness at the same time. Insulin provides another such example. Many diabetic patients need to maintain a constant baseline level of insulin which is different from their needs at the time of meals. The baseline level may be maintained using transdermal administration of insulin, as described herein. Antibiotics may also be administered at a constant rate, maintaining adequate

25 bactericidal blood levels, while avoiding the high levels that are often responsible for the toxicity (*e.g.*, levels of gentamycin that are too high typically result in renal toxicity).

Drug delivery by the methods of the present invention also provide a more convenient method of drug administration. For example, it is often particularly

30 difficult to administer parenteral drugs to newborns and infants because of the difficulty associated with finding veins of acceptable caliber to catheterize. However, newborns

and infants often have a relatively large skin surface as compared to adults. Transdermal drug delivery permits easier management of such patients and allows certain types of care that can presently be given only in hospitals to be given at home. Other patients who typically have similar difficulties with venous catheterization are

5   patients undergoing chemotherapy or patients on dialysis. In addition, for patients undergoing prolonged therapy, transdermal administration as described herein is more convenient than parenteral administration.

Transdermal administration as described herein also allows the gastrointestinal tract to be bypassed in situations where parenteral uses would not be

10   practical. For example, there is a growing need for methods suitable for administration of therapeutic small peptides and proteins, which are typically digested within the gastrointestinal tract. The methods described herein permit administration of such compounds and allow easy administration over long periods of time. Patients who have problems with absorption through their gastrointestinal tract because of prolonged ileus

15   or specific gastrointestinal diseases limiting drug absorption may also benefit from drugs formulated for transdermal application as described herein.

Further, there are many clinical situations where it is difficult to maintain compliance. For example, patients with mental problems (e.g., patients with Alzheimer's disease or psychosis) are easier to manage if a constant delivery rate of drug

20   is provided without having to rely on their ability to take their medication at specific times of the day. Also patients who simply forget to take their drugs as prescribed are less likely to do so if they merely have to put on a skin patch periodically (e.g., every 3 days). Patients with diseases that are without symptoms, like patients with hypertension, are especially at risk of forgetting to take their medication as prescribed.

25   For patients taking multiple drugs, devices for transdermal application such as skin patches may be formulated with combinations of drugs that are frequently used together. For example, many heart failure patients are given digoxin in combination with furosemide. The combination of both drugs into a single skin patch facilitates administration, reduces the risk of errors (taking the correct pills at the

30   appropriate time is often confusing to older people), reduces the psychological strain of

taking "so many pills," reduces skipped dosage because of irregular activities and improves compliance.

The methods described herein are particularly applicable to humans, but also have a variety of veterinary uses, such as the administration of growth factors or

5   hormones (*e.g.*, for fertility control) to an animal.

As noted above, a wide variety of drugs may be administered according to the methods provided herein. Some examples of drug categories that may be administered transdermally include anti-inflammatory drugs (*e.g.*, in arthritis and in other condition) such as all NSAID, indomethacin, prednisone, etc.; analgesics

10 (especially when oral absorption is not possible, such as after surgery, and when parenteral administration is not convenient or desirable), including morphine, codeine, Demerol, acetaminophen and combinations of these (*e.g.*, codeine plus acetaminophen); antibiotics such as Vancomycin (which is not absorbed by the GI tract and is frequently given intravenously) or a combination of INH and Rifampicin (*e.g.*,

15 for tuberculosis); anticoagulants such as heparin (which is not well absorbed by the GI tract and is generally given parenterally, resulting in fluctuation in the blood levels with an increased risk of bleeding at high levels and risks of inefficacy at lower levels) and Warfarin (which is absorbed by the GI tract but cannot be administered immediately after abdominal surgery because of the normal ileus following the procedure);

20 antidepressants (*e.g.*, in situations where compliance is an issue as in Alzheimer's disease or when maintaining stable blood levels results in a significant reduction of anti-cholinergic side effects and better tolerance by patients), such as amitriptylin, imipramin, prozac, etc.; antihypertensive drugs (*e.g.*, to improve compliance and reduce side effects associated with fluctuating blood levels), such as diuretics and beta-blockers

25 (which can be administered by the same patch; *e.g.*, furosemide and propanolol); antipsychotics (*e.g.*, to facilitate compliance and make it easier for care giver and family members to make sure that the drug is received), such as haloperidol and chlorpromazine; and anxiolytics or sedatives (*e.g.*, to avoid the reduction of alertness related to high blood levels after oral administration and allow a continual benefit

30 throughout the day by maintaining therapeutic levels constant).

Numerous other drugs may be administered as described herein, including naturally occurring and synthetic hormones, growth factors, proteins and peptides. For example, insulin and human growth hormone, growth factors like erythropoietin, interleukins and interferons may be delivered via the skin.

5   Kits for administering a drug via the skin of a mammal are also provided within the present invention. Such kits generally comprise a device for transdermal application (*i.e.,* skin patch) in combination with, or impregnated with, one or more cyclic peptides. A drug may additionally be included within such kits.

Within a related embodiment, the use of cyclic peptides as described
10   herein to increase skin permeability may also facilitate sampling of the blood compartment by passive diffusion, permitting detection and/or measurement of the levels of specific molecules circulating in the blood. For example, application of one or more cyclic peptides to the skin, via a skin patch as described herein, permits the patch to function like a sponge to accumulate a small quantity of fluid containing a
15   representative sample of the serum. The patch is then removed after a specified amount of time and analyzed by suitable techniques for the compound of interest (*e.g.,* a medication, hormone, growth factor, metabolite or marker). Alternatively, a patch may be impregnated with reagents to permit a color change if a specific substance (*e.g.,* an enzyme) is detected. Substances that can be detected in this manner include, but are not
20   limited to, illegal drugs such as cocaine, HIV enzymes, glucose and PSA. This technology is of particular benefit for home testing kits.

Within a further aspect, methods are provided for enhancing delivery of a drug to a tumor in a mammal, comprising administering a cyclic peptide in combination with a drug to a tumor-bearing mammal. Cyclic peptides for use within such methods
25   include those designed to disrupt E-cadherin and/or N-cadherin mediated cell adhesion, and include N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8), N-Ac-CHAVSC-NH$_2$ (SEQ ID NO:14), N-Ac-CAHAVDC-NH$_2$ (SEQ ID NO:16), N-Ac-CSHAVSSC-NH$_2$ (SEQ ID NO:18) and N-Ac-KHAVD-NH$_2$ (SEQ ID NO:20). Preferably, the cyclic peptide and the drug are formulated within the same composition or drug delivery device prior to
30   administration. In general, a cyclic peptide may enhance drug delivery to any tumor, and the method of administration may be chosen based on the type of target tumor. For

example, injection or topical administration as described above may be preferred for melanomas and other accessible tumors (*e.g.*, metastases from primary ovarian tumors may be treated by flushing the peritoneal cavity with the composition). Other tumors (*e.g.*, bladder tumors) may be treated by injection of the cyclic peptide and the drug

5   (such as mitomycin C) into the site of the tumor. In other instances, the composition may be administered systemically, and targeted to the tumor using any of a variety of specific targeting agents. Suitable drugs may be identified by those of ordinary skill in the art based upon the type of cancer to be treated (*e.g.*, mitomycin C for bladder cancer). In general, the amount of cyclic peptide administered varies with the method

10   of administration and the nature of the tumor, within the typical ranges provided above, preferably ranging from about 1μg/mL to about 2 mg/mL, and more preferably from about 10μg/mL to 100μg/mL. Transfer of the drug to the target tumor may be evaluated by appropriate means that will be apparent to those of ordinary skill in the art, such as a reduction in tumor size. Drugs may also be labeled (*e.g.*, using radionuclides) to permit

15   direct observation of transfer to the target tumor using standard imaging techniques.

Within a related aspect, the present invention provides methods for treating cancer and/or inhibiting metastasis in a mammal. Cancer tumors are solid masses of cells, growing out of control, which require nourishment via blood vessels. The formation of new capillaries is a prerequisite for tumor growth and the emergence

20   of metastases. Administration of cyclic peptides as described herein may disrupt the growth of such blood vessels, thereby providing effective therapy for the cancer and/or inhibiting metastasis. Cyclic peptides may also be used to treat leukemias. Preferred cyclic peptides for use within such methods include those that disrupt N-cadherin mediated cell adhesion, such as N-Ac-CHAVC-NH2 (SEQ ID NO:8), N-Ac-CHAVSC-

25   NH2 (SEQ ID NO:14), N-Ac-CAHAVDC-NH2 (SEQ ID NO:16), N-Ac-CSHAVSSC-NH2 (SEQ ID NO:18) and N-Ac-KHAVD-NH2 (SEQ ID NO:20). A cyclic peptide may be administered alone (*e.g.*, via the skin) or within a pharmaceutical composition. For melanomas and certain other accessible tumors, injection or topical administration as described above may be preferred. For ovarian cancers, flushing the peritoneal cavity

30   with a composition comprising one or more cyclic peptides may prevent metastasis of ovarian tumor cells. Other tumors (*e.g.*, bladder tumors, bronchial tumors or tracheal

tumors) may be treated by injection of the cyclic peptide into the cavity. In other instances, the composition may be administered systemically, and targeted to the tumor using any of a variety of specific targeting agents, as described above. In general, the amount of cyclic peptide administered varies depending upon the method of administration and the nature of the cancer, but may vary within the ranges identified above. The effectiveness of the cancer treatment or inhibition of metastasis may be evaluated using well known clinical observations such as the level of serum markers (*e.g.*, CEA or PSA).

Within a further related aspect, a cyclic peptide may be used to inhibit angiogenesis (*i.e.*, the growth of blood vessels from pre-existing blood vessels) in a mammal. In general, inhibition of angiogenesis may be beneficial in patients afflicted with diseases such as cancer or arthritis. Preferred cyclic peptides for inhibition of angiogenesis include H-CHAVC-NH$_2$ (SEQ ID NO:8) and N-Ac-CHAVSC-NH$_2$ (SEQ ID NO:14). The effect of a particular cyclic peptide on angiogenesis may generally be determined by evaluating the effect of the peptide on blood vessel formation. Such a determination may generally be performed, for example, using a chick chorioallantoic membrane assay (Iruela-Arispe et al., *Molecular Biology of the Cell 6*:327-343, 1995). Briefly, a cyclic peptide may be embedded in a mesh composed of vitrogen at one or more concentrations (*e.g.*, ranging from about 1 to 100 µg/mesh). The mesh(es) may then be applied to chick chorioallantoic membranes. After 24 hours, the effect of the peptide may be determined using computer assisted morphometric analysis. A cyclic peptide should inhibit angiogenesis by at least 25% at a concentration of 33 µg/mesh.

The addition of a targeting agent may be beneficial, particularly when the administration is systemic. Suitable modes of administration and dosages depend upon the condition to be prevented or treated but, in general, administration by injection is appropriate. Dosages may vary as described above. The effectiveness of the inhibition may be evaluated grossly by assessing the inability of the tumor to maintain growth and microscopically by an absence of nerves at the periphery of the tumor.

In yet another related aspect, the present invention provides methods for inducing apoptosis in a cadherin-expressing cell. In general, patients afflicted with cancer may benefit from such treatment. Preferred cyclic peptides for use within such

methods include N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8), N-Ac-CHAVSC-NH$_2$ (SEQ ID NO:14), N-Ac-CAHAVDC-NH$_2$ (SEQ ID NO:16), N-Ac-CSHAVSSC-NH$_2$ (SEQ ID NO:18) and N-Ac-KHAVD-NH$_2$ (SEQ ID NO:20). Cyclic peptides linked to a molecule comprising a binding site for a second adhesion molecule (such as Arg-Gly-Asp) by way of a linker are also preferred. Administration may be topical, via injection or by other means, and the addition of a targeting agent may be beneficial, particularly when the administration is systemic. Suitable modes of administration and dosages depend upon the location and nature of the cells for which induction of apoptosis is desired but, in general, dosages may vary as described above. A biopsy may be performed to evaluate the level of induction of apoptosis.

The present invention also provides methods for enhancing drug delivery to the central nervous system of a mammal. The blood/brain barrier is largely impermeable to most neuroactive agents, and delivery of drugs to the brain of a mammal often requires invasive procedures. Using a cyclic peptide as described herein, however, delivery may be by, for example, systemic administration of a cyclic peptide-drug-targeting agent combination, injection of a cyclic peptide (alone or in combination with a drug and/or targeting agent) into the carotid artery or application of a skin patch comprising a cyclic peptide to the head of the patient. Certain preferred cyclic peptides for use within such methods are relatively small (e.g., a ring size of 4-10 residues; preferably 5-7 residues) and include peptides comprising a 5-residue ring such as N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8) and N-Ac-KHAVD-NH$_2$ (SEQ ID NO:20). In general, the amount of cyclic peptide administered varies with the method of administration and the nature of the condition to be treated or prevented, but typically varies as described above. Transfer of the drug to the central nervous system may be evaluated by appropriate means that will be apparent to those of ordinary skill in the art, such as magnetic resonance imaging (MRI) or PET scan (positron emitted tomography).

In still further aspects, the present invention provides methods for enhancing adhesion of cadherin-expressing cells. Within such embodiments, a cyclic peptide is generally linked to a solid support as described above. The resulting matrix, which comprises multiple linked cyclic peptides can be used as a "biological glue" to bind multiple cadherin-expressing cells within a variety of contexts.

Within one embodiment, matrix-linked cyclic peptides may be used to enhance wound healing and/or reduce scar tissue in a mammal. Preferred cyclic peptides for use within such methods include N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8), N-Ac-KHAVD-NH$_2$ (SEQ ID NO:20), and cyclic peptides that are linked to a biocompatible and biodegradable matrix such as cellulose or collagen are particularly preferred. For use within such methods, a cyclic peptide should have a free amino or hydroxyl group. The peptides are generally administered topically to the wound, where they may facilitate closure of the wound and may augment, or even replace, stitches. Similarly, administration of matrix-linked cyclic peptides may facilitate cell adhesion in skin grafting and prosthetic implants and may prolong the duration and usefulness of collagen injection. In general, the amount of matrix-linked cyclic peptide administered to a wound, graft or implant site varies with the severity of the wound and/or the nature of the wound, graft, or implant, but may vary as discussed above.

Within another embodiment, one or more cyclic peptides may be linked to the interior surface of a tissue culture plate or other cell culture support, such as for use in a bioreactor. Such linkage may be performed by any suitable technique, as described above. Cyclic peptides linked in this fashion may generally be used to immobilize cadherin-expressing cells. For example, dishes or plates coated with one or more cyclic peptides may be used to immobilize cadherin-expressing cells within a variety of assays and screens. Within bioreactors (*i.e.*, systems for larger scale production of cells or organoids), cyclic peptides may generally be used to improve cell attachment and stabilize cell growth. Cyclic peptides may also be used within bioreactors to support the formation and function of highly differentiated organoids derived, for example, from dispersed populations of fetal mammalian cells. Bioreactors containing biomatrices of cyclic peptide(s) may also be used to facilitate the production of specific proteins.

Cyclic peptides as described herein may be used within a variety of bioreactor configurations. In general, a bioreactor is designed with an interior surface area sufficient to support larger numbers of adherent cells. This surface area can be provided using membranes, tubes, microtiter wells, columns, hollow fibers, roller bottles, plates, dishes, beads or a combination thereof. A bioreactor may be

compartmentalized. The support material within a bioreactor may be any suitable material known in the art; preferably, the support material does not dissolve or swell in water. Preferred support materials include, but are not limited to, synthetic polymers such as acrylics, vinyls, polyethylene, polypropylene, polytetrafluoroethylene, nylons,

5 polyurethanes, polyamides, polysulfones and poly(ethylene terephthalate); ceramics; glass and silica.

Cyclic peptides may also be used, within other aspects of the present invention, to enhance and/or direct neurological growth. In one aspect, neurite outgrowth may be enhanced and/or directed by contacting a neuron with one or more

10 cyclic peptides. Preferred cyclic peptides for use within such methods are linked to a polymeric matrix or other support and include those peptides without substantial flanking sequences, as described above. In particularly preferred embodiments, the cyclic peptide is N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8) or N-Ac-KHAVD-NH$_2$ (20). Within other preferred embodiments, a cyclic peptide is linked to a molecule

15 comprising an integrin cell recognition sequence (Arg-Gly-Asp), as described above. The method of achieving contact and the amount of cyclic peptide used will depend upon the location of the neuron and the extent and nature of the outgrowth desired. For example, a neuron may be contacted (*e.g.*, via implantation) with cyclic peptide(s) linked to a support material such as a suture, fiber nerve guide or other prosthetic device

20 such that the neurite outgrowth is directed along the support material. Alternatively, a tubular nerve guide may be employed, in which the lumen of the nerve guide contains a composition comprising the cyclic peptide(s). *In vivo*, such nerve guides or other supported cyclic peptides may be implanted using well known techniques to, for example, facilitate the growth of severed neuronal connections and/or to treat spinal

25 cord injuries. It will be apparent to those of ordinary skill in the art that the structure and composition of the support should be appropriate for the particular injury being treated. *In vitro*, a polymeric matrix may similarly be used to direct the growth of neurons onto patterned surfaces as described, for example, in U.S. Patent No. 5,510,628.

30 Within another such aspect, one or more cyclic peptides may be used for therapy of a demyelinating neurological disease in a mammal. It has been found, within

the context of the present invention that Schwann cell migration on astrocytes is inhibited by N-cadherin. There are a number of neurological diseases, such as multiple sclerosis, where oligodendrocytes die. In theory, Schwann cells from the peripheral nervous system could be used to replace damaged oligodendrocytes in the CNS. However, previous attempts to perform such a replacement have failed, because Schwann cells implanted into the brain have not migrated on astrocytes or remyelinated damaged neurons. Cyclic peptides as described herein, when injected with Schwann cells into the brain, may facilitate Schwann cell migration and permit the practice of such therapy.

Multiple sclerosis patients suitable for treatment may be identified by criteria that establish a diagnosis of clinically definite or clinically probable MS (*see* Poser et al., *Ann. Neurol. 13*:227, 1983). Candidate patients for preventive therapy may be identified by the presence of genetic factors, such as HLA-type DR2a and DR2b, or by the presence of early disease of the relapsing remitting type.

Schwann cell grafts may be implanted directly into the brain along with the cyclic peptide(s) using standard techniques. Preferred cyclic peptides for use within such methods include N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8) and N-Ac-KHAVD-NH$_2$ (SEQ ID NO:20). Suitable amounts of cyclic peptide generally range as described above, preferably from about 10µg/mL to about 1 mg/mL.

Alternatively, a cyclic peptide may be administered alone or within a pharmaceutical composition. The duration and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease. Within particularly preferred embodiments of the invention, the cyclic peptide or pharmaceutical composition may be administered at a dosage ranging from 0.1 mg/kg to 20 mg/kg although appropriate dosages may be determined by clinical trials. Methods of administration include injection, intravenous or intrathecal (*i.e.,* directly in cerebrospinal fluid).

Effective treatment of multiple sclerosis may be evidenced by any of the following criteria: EDSS (extended disability status scale), appearance of exacerbations or MRI (magnetic resonance imaging). The EDSS is a means to grade clinical impairment due to MS (Kurtzke, *Neurology 33*:1444, 1983), and a decrease of one full

step defines an effective treatment in the context of the present invention (Kurtzke, *Ann. Neurol. 36*:573-79, 1994). Exacerbations are defined as the appearance of a new symptom that is attributable to MS and accompanied by an appropriate new neurologic abnormality (Sipe et al., *Neurology 34*:1368, 1984). Therapy is deemed to be effective

5   if there is a statistically significant difference in the rate or proportion of exacerbation-free patients between the treated group and the placebo group or a statistically significant difference in the time to first exacerbation or duration and severity in the treated group compared to control group. MRI can be used to measure active lesions using gadolinium-DTPA-enhanced imaging (McDonald et al. *Ann. Neurol. 36*:14, 1994)

10   or the location and extent of lesions using T_2-weighted techniques. The presence, location and extent of MS lesions may be determined by radiologists using standard techniques. Improvement due to therapy is established when there is a statistically significant improvement in an individual patient compared to baseline or in a treated group versus a placebo group.

15   Efficacy of the cyclic peptide in the context of prevention may be judged based on clinical measurements such as the relapse rate and EDSS. Other criteria include a change in area and volume of T2 images on MRI, and the number and volume of lesions determined by gadolinium enhanced images.

           Within another aspect, cyclic peptides as described herein may be used

20   for modulating the immune system of a mammal in any of several ways. Cadherins are expressed on immature B and T cells (thymocytes and bone marrow pre-B cells), as well as on specific subsets of activated B and T lymphocytes and some hematological malignancies (*see* Lee et al., *J. Immunol. 152*:5653-5659, 1994; Munro et al., *Cellular Immunol. 169*:309-312, 1996; Tsutsui et al., *J. Biochem. 120*:1034-1039, 1996; Cepek

25   et al., Proc. Natl. Acad. Sci. USA 93:6567-6571, 1996). Cyclic peptides may generally be used to modulate specific steps within cellular interactions during an immune response or during the dissemination of malignant lymphocytes.

           For example, a cyclic peptide as described herein may be used to treat diseases associated with excessive generation of otherwise normal T cells. Without

30   wishing to be bound by any particular theory, it is believed that the interaction of cadherins on maturing T cells and B cell subsets contributes to protection of these cells

from programmed cell death. A cyclic peptide may decrease such interactions, leading to the induction of programmed cell death. Accordingly, cyclic peptides may be used to treat certain types of diabetes and rheumatoid arthritis, particularly in young children where the cadherin expression on thymic pre-T cells is greatest.

5   Cyclic peptides may also be administered to patients afflicted with certain skin disorders (such as cutaneous lymphomas), acute B cell leukemia and excessive immune reactions involving the humoral immune system and generation of immunoglobulins, such as allergic responses and antibody-mediated graft rejection. In addition, patients with circulating cadherin-positive malignant cells (*e.g.,* during

10   regimes where chemotherapy or radiation therapy is eliminating a major portion of the malignant cells in bone marrow and other lymphoid tissue) may benefit from treatment with a cyclic peptide. Such treatment may also benefit patients undergoing transplantation with peripheral blood stem cells.

Preferred cyclic peptides for use within such methods include those that

15   disrupt E-cadherin and/or N-cadherin mediated cell adhesion, such as N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8), N-Ac-CHAVSC-NH$_2$ (SEQ ID NO:14), N-Ac-CSHAVSSC-NH$_2$ (SEQ ID NO:18) and N-Ac-KHAVD-NH$_2$ (SEQ ID NO:20). Within the above methods, the cyclic peptide(s) are preferably administered systemically (usually by injection) or topically. A cyclic peptide may be linked to a targeting agent. For

20   example, targeting to the bone marrow may be beneficial. A suitable dosage is sufficient to effect a statistically significant reduction in the population of B and/or T cells that express cadherin and/or an improvement in the clinical manifestation of the disease being treated. Typical dosages range as described above.

Within further aspects, the present invention provides methods and kits

25   for preventing pregnancy in a mammal. In general, disruption of E-cadherin function prevents the adhesion of trophoblasts and their subsequent fusion to form syncitiotrophoblasts. In one embodiment, one or more cyclic peptides as described herein may be incorporated into any of a variety of well known contraceptive devices, such as sponges suitable for intravaginal insertion (*see, e.g.,* U.S. Patent No. 5,417,224)

30   or capsules for subdermal implantation. Other modes of administration are possible, however, including transdermal administration, for cyclic peptides linked to an

appropriate targeting agent. Preferred cyclic peptides for use within such methods include N-Ac-CHAVC-NH$_2$ (SEQ ID NO:8), N-Ac-CHAVSC-NH$_2$ (SEQ ID NO:14), N-Ac-CSHAVSSC-NH$_2$ (SEQ ID NO:18) and N-Ac-KHAVD-NH$_2$ (SEQ ID NO:20). Suitable methods for incorporation into such a device depend upon the type of device

5   and are well known in the art. Such devices facilitate administration of the cyclic peptide(s) to the uterine region and may provide a sustained release of the cyclic peptide(s). In general, cyclic peptide(s) may be administered via such a contraceptive device at a dosage ranging from 0.1 to 20 mg/kg, although appropriate dosages may be determined by monitoring hCG levels in the urine. hCG is produced by the placenta,

10 and levels of this hormone rise in the urine of pregnant women. The urine hCG levels can be assessed by radio-immunoassay using well known techniques. Kits for preventing pregnancy generally comprise a contraceptive device impregnated with one or more cyclic peptides.

Alternatively, a sustained release formulation of one or more cyclic

15 peptides may be implanted, typically subdermally, in a mammal for the prevention of pregnancy. Such implantation may be performed using well known techniques. Preferably, the implanted formulation provides a dosage as described above, although the minimum effective dosage may be determined by those of ordinary skill in the art using, for example, an evaluation of hCG levels in the urine of women.

20 The present invention also provides methods for increasing vasopermeability in a mammal by administering one or more cyclic peptides or pharmaceutical compositions. Within blood vessels, endothelial cell adhesion (mediated by N-cadherin) results in decreased vascular permeability. Accordingly, cyclic peptides as described herein may be used to increase vascular permeability.

25 Within certain embodiments, preferred cyclic peptides for use within such methods include peptides capable of decreasing both endothelial and tumor cell adhesion. Such peptides may be used to facilitate the penetration of anti-tumor therapeutic or diagnostic agents (e.g., monoclonal antibodies) through endothelial cell permeability barriers and tumor barriers. Particularly preferred peptides include N-Ac-CHAVC-NH$_2$ (SEQ ID

30 NO:8) and N-Ac-KHAVD-NH$_2$ (SEQ ID NO:20).

Treatment with a cyclic peptide may be appropriate, for example, prior to administration of an anti-tumor therapeutic or diagnostic agent (*e.g.,* a monoclonal antibody or other macromolecule), an antimicrobial agent or an anti-inflammatory agent, in order to increase the concentration of such agents in the vicinity of the target

5 tumor, organism or inflammation without increasing the overall dose to the patient. Cyclic peptides for use within such methods may be linked to a targeting agent to further increase the local concentration of cyclic peptide, although systemic administration of a vasoactive agent even in the absence of a targeting agent increases the perfusion of certain tumors relative to other tissues. Suitable targeting agents

10 include antibodies and other molecules that specifically bind to tumor cells or to components of structurally abnormal blood vessels. For example, a targeting agent may be an antibody that binds to a fibrin degradation product or a cell enzyme such as a peroxidase that is released by granulocytes or other cells in necrotic or inflamed tissues.

Administration via intravenous injection or transdermal administration is

15 generally preferred. Effective dosages are generally sufficient to increase localization of a subsequently administered diagnostic or therapeutic agent to an extent that improves the clinical efficacy of therapy of accuracy of diagnosis to a statistically significant degree. Comparison may be made between treated and untreated tumor host animals to whom equivalent doses of the diagnostic or therapeutic agent are administered. In

20 general, dosages range as described above.

Other aspects of the present invention provide methods that exploit the immunogenic properties of the cyclic peptides. For example, polyclonal and monoclonal antibodies may be raised against a cyclic peptide using conventional techniques. Such antibodies may be prepared by any of a variety of techniques known to

25 those of ordinary skill in the art. *See, e.g.,* Harlow and Lane, *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory, 1988. In one such technique, an immunogen comprising the cyclic peptide is initially injected into any of a wide variety of mammals (*e.g.,* mice, rats, rabbits, sheep or goats). Because of its small size, the cyclic peptide should be joined to a carrier protein, such as bovine serum albumin or keyhole limpet

30 hemocyanin. Following one or more injections, the animals are bled periodically. Polyclonal antibodies specific for the cyclic peptide may then be purified from such

antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for the cyclic peptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* 5 *6*:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity from spleen cells obtained from an animal immunized as described above. The spleen cells are immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. Single colonies 10 are selected and their culture supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies, with or without the use of various techniques known in the art to enhance the yield. Contaminants may be removed from the antibodies by conventional 15 techniques, such as chromatography, gel filtration, precipitation, and extraction. Antibodies having the desired activity may generally be identified using immunofluorescence analyses of tissue sections, cell or other samples where the target cadherin is localized.

Such antibodies may be used *in vitro* or *in vivo* to modulate cell 20 adhesion. Within certain embodiments, antibodies may be used within methods in which enhanced cell adhesion is desired, as described above. For example, antibodies may be used within the above methods for enhancing and/or directing neurite outgrowth *in vitro* or *in vivo*. Antibodies may be used within the lumen of a tubular nerve guide or may be attached to a fiber nerve guide, suture or other solid support and used as 25 described above for cyclic peptides. Antibody dosages are sufficient to enhance or direct neurite outgrowth, and will vary with the method of administration and the condition to be treated.

Antibodies may also be used as a "biological glue, " as described above to bind multiple cadherin-expressing cells within a variety of contexts, such as to 30 enhance wound healing and/or reduce scar tissue, and/or to facilitate cell adhesion in skin grafting or prosthetic implants. In general, the amount of matrix-linked antibody

administered to a wound, graft or implant site varies with the severity of the wound and/or the nature of the wound, graft, or implant, but may vary as discussed above. Antibodies may also be linked to any of a variety of support materials, as described above, for use in tissue culture or bioreactors.

5   Within certain embodiments, antibodies (or, preferably, antigen-binding fragments thereof) may be used in situations where inhibition of cell adhesion is desired. Such antibodies or fragments may be used, for example, for treatment of demyelinating diseases, such as MS, or to inhibit interactions between tumor cells, as described above. The use of Fab fragments is generally preferred.

10   Cyclic peptides may also be used to generate monoclonal antibodies, as described above, that are specific for particular cadherins (e.g., antibodies that bind to E-cadherin, but do not bind significantly to N-cadherin, or vise versa). Such antibodies may generally be used for therapeutic, diagnostic and assay purposes. For example, such antibodies may be linked to a drug and administered to a mammal to target the

15   drug to a particular cadherin-expressing cell, such as a leukemic cell in the blood.

Assays typically involve using an antibody to detect the presence or absence of a cadherin (free or on the surface of a cell), or proteolytic fragment containing the EC1 domain in a suitable biological sample, such as tumor or normal tissue biopsies, blood, lymph node, serum or urine samples, or other tissue,

20   homogenate, or extract thereof obtained from a patient.

There are a variety of assay formats known to those of ordinary skill in the art for using an antibody to detect a target molecule in a sample. See, e.g., Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988. For example, the assay may be performed in a Western blot format, wherein a protein

25   preparation from the biological sample is submitted to gel electrophoresis, transferred to a suitable membrane and allowed to react with the antibody. The presence of the antibody on the membrane may then be detected using a suitable detection reagent, as described below.

In another embodiment, the assay involves the use of antibody

30   immobilized on a solid support to bind to the target cadherin, or a proteolytic fragment containing the EC1 domain, and remove it from the remainder of the sample. The

bound cadherin may then be detected using a second antibody or reagent that contains a reporter group. Alternatively, a competitive assay may be utilized, in which a cadherin is labeled with a reporter group and allowed to bind to the immobilized antibody after incubation of the antibody with the sample. The extent to which components of the

5   sample inhibit the binding of the labeled cadherin to the antibody is indicative of the reactivity of the sample with the immobilized antibody, and as a result, indicative of the level of the cadherin in the sample.

The solid support may be any material known to those of ordinary skill in the art to which the antibody may be attached, such as a test well in a microtiter plate,

10 a nitrocellulose filter or another suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic such as polystyrene or polyvinylchloride. The antibody may be immobilized on the solid support using a variety of techniques known to those in the art, which are amply described in the patent and scientific literature.

15 In certain embodiments, the assay for detection of a cadherin in a sample is a two-antibody sandwich assay. This assay may be performed by first contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the biological sample, such that the cadherin within the sample is allowed to bind to the immobilized antibody (a 30 minute incubation time at room

20 temperature is generally sufficient). Unbound sample is then removed from the immobilized cadherin-antibody complexes and a second antibody (containing a reporter group such as an enzyme, dye, radionuclide, luminescent group, fluorescent group or biotin) capable of binding to a different site on the cadherin is added. The amount of second antibody that remains bound to the solid support is then determined using a

25 method appropriate for the specific reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group

30 (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of

time), followed by spectroscopic or other analysis of the reaction products. Standards and standard additions may be used to determine the level of cadherin in a sample, using well known techniques.

The present invention also provides kits for use in such immunoassays. Such kits generally comprise one or more antibodies, as described above. In addition, one or more additional compartments or containers of a kit generally enclose elements, such as reagents, buffers and/or wash solutions, to be used in the immunoassay.

Within further aspects, cyclic peptides or antibodies thereto may be used to facilitate cell identification and sorting *in vitro* or imaging *in vivo*, permitting the selection of cells expressing different cadherins (or different cadherin levels). Preferably, the cyclic peptide(s) or antibodies for use in such methods are linked to a detectable marker. Suitable markers are well known in the art and include radionuclides, luminescent groups, fluorescent groups, enzymes, dyes, constant immunoglobulin domains and biotin. Within one preferred embodiment, a cyclic peptide or antibody linked to a fluorescent marker, such as fluorescein, is contacted with the cells, which are then analyzed by fluorescence activated cell sorting (FACS).

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

Preparation of Representative Cyclic Peptides

This Example illustrates the solid phase synthesis of representative cyclic

5   peptides.

The peptides were assembled on methylbenzhydrylamine resin (MBHA resin) for the C-terminal amide peptides. The traditional Merrifield resins were used for any C-terminal acid peptides. Bags of a polypropylene mesh material were filled with the resin and soaked in dichloromethane. The resin packets were washed three times

10   with 5% diisopropylethylamine in dichloromethane and then washed with dichloromethane. The packets are then sorted and placed into a Nalgene bottle containing a solution of the amino acid of interest in dichloromethane. An equal amount of diisopropylcarbodiimide (DIC) in dichloromethane was added to activate the coupling reaction. The bottle was shaken for one hour to ensure completion of the

15   reaction. The reaction mixture was discarded and the packets washed with DMF. The N-α-Boc was removed by acidolysis using a 55% TFA in dichloromethane for 30 minutes leaving the TFA salt of the α-amino group. The bags were washed and the synthesis completed by repeating the same procedure while substituting for the corresponding amino acid at the coupling step. Acetylation of the N-terminal was

20   performed by reacting the peptide resins with a solution of acetic anhydride in dichloromethane in the presence of diisopropylethylamine. The peptide was then side-chain deprotected and cleaved from the resin at 0°C with liquid HF in the presence of anisole as a carbocation scavenger.

The crude peptides were purified by reversed-phase high-performance

25   liquid chromatography. Purified linear precursors of the cyclic peptides were solubilized in 75% acetic acid at a concentration of 2-10mg/mL. A 10% solution of iodine in methanol was added dropwise until a persistent coloration was obtained. A 5% ascorbic acid solution in water was then added to the mixture until discoloration. The disulfide bridge containing compounds were then purified by HPLC and characterized

30   by analytical HPLC and by mass spectral analysis.

EXAMPLE 2

Disruption of the Ability of Mouse Cerebellar Neurons to Extend Neurites

5   Three cell adhesion molecules, N-cadherin, N-CAM and L1, are capable of regulating neurite outgrowth (Doherty and Walsh, *Curr. Op. Neurobiol.* 4:49-55, 1994; Williams et al., *Neuron* 13:583-594, 1994; Hall et al., *Cell Adhesion and Commun.* 3:441-450, 1996; Doherty and Walsh, *Mol. Cell. Neurosci.* 8:99-111, 1994; Safell et al., *Neuron* 18:231-242, 1997). Neurons cultured on monolayers of 3T3 cells

10   that have been transfected with cDNAs encoding N-cadherin, N-CAM or L1 extend longer neurites than neurons cultured on 3T3 cells not expressing these cell adhesion molecules. This Example illustrates the use of a representative cyclic peptide to inhibit neurite outgrowth.

        Neurons were cultured on monolayers of 3T3 cells transfected with

15   cDNA encoding N-cadherin essentially as described by Doherty and Walsh, *Curr. Op. Neurobiol.* 4:49-55, 1994; Williams et al., *Neuron* 13:583-594, 1994; Hall et al., *Cell Adhesion and Commun.* 3:441-450, 1996; Doherty and Walsh, *Mol. Cell. Neurosci.* 8:99-111, 1994; Safell et al., *Neuron* 18:231-242, 1997. Briefly, monolayers of control 3T3 fibroblasts and 3T3 fibroblasts that express N-cadherin were established by

20   overnight culture of 80,000 cells in individual wells of an 8-chamber well tissue culture slide. 3000 cerebellar neurons isolated from post-natal day 3 mouse brains were cultured for 18 hours on the various monolayers in control media (SATO/2%FCS), or media supplemented with various concentrations of the cyclic peptide N-Ac-CHAVC-NH$_2$ or a control peptide without the HAV sequence (N-Ac-CHGVC-NH$_2$). The

25   cultures were then fixed and stained for GAP43 which specifically binds to the neurons and their neurites. The length of the longest neurite on each GAP43 positive neuron was then measured by computer assisted morphometry.

        As shown in Figure 4, culture for 18 hours with N-Ac-CHAVC-NH$_2$ at a concentration of 500 µg/mL inhibited neurite outgrowth on 3T3 cells expressing N-

30   cadherin, whereas the cyclic peptide N-Ac-CHGVC-NH$_2$ (also at a concentration of 500 µg/ml) had no effect on this process. Furthermore, the cyclic peptide N-Ac-CHAVC-

NH$_2$ (used at a concentration of 500 µg/ml) did not inhibit neurite outgrowth on 3T3 cells not expressing N-cadherin, N-CAM, or L1 (control cells), thus indicating that the peptide is not toxic and that it has no non-specific effects on neurite outgrowth (Figure 4, compare columns 3 and 1). These data also indicate that the peptide does not effect

5   integrin function.

A dose-response study demonstrated that N-Ac-CHAVC-NH$_2$ significantly inhibited neurite outgrowth on 3T3 cells expressing N-cadherin at a concentration of 50 µg/mL, and completely inhibited neurite outgrowth on these cells at a concentration of 500 µg/mL (Figure 5). Finally, N-Ac-CHAVC-NH$_2$ (used at a

10   concentration of 500 µg/mL) did not inhibit neurite outgrowth on 3T3 cells expressing either N-CAM or L1 (Figure 6). These results indicate that the peptide N-Ac-CHAVC-NH$_2$ specifically inhibits the function of N-cadherin. Collectively, the results obtained from these studies demonstrate that N-Ac-CHAVC-NH$_2$ is an effective inhibitor of neurite outgrowth by virtue of its ability to disrupt N-cadherin function.

15

EXAMPLE 3

Disruption of Bovine Endothelial Cell Adhesion

20   This Example illustrates the use of representative cyclic peptides to disrupt adhesion of endothelial cells, which express N-cadherin.

Bovine pulmonary artery endothelial cells were harvested by sterile ablation and digestion in 0.1% collagenase (type II; Worthington Enzymes, Freehold, NJ). Cells were maintained in Dulbecco's minimum essential medium (Clonetics, San

25   Diego, CA) supplemented with 10% fetal calf serum (Atlantic biologicals, Nor cross, GA) and 1% antibiotic-antimycotic at 37°C in 7% CO$_2$ in air. Cultures were passaged weekly in trypsin-EDTA (Gibco, Grand Island, NY) and seeded onto tissue culture plastic at 20,000 cells/cm^2 for all experiments. Endothelial cultures were used at 1 week in culture, which is approximately 3 days after culture confluency was established. The

30   cells used in all protocols were between 4th passage and 10th passage. The cells were

seeded onto coverslips and treated 30 minutes with N-Ac-CHAVC-NH$_2$ or N-Ac-CHGVC-NH$_2$ at 500µg/ml and then fixed with 1% paraformaldehyde.

The peptide N-Ac-CHAVC-NH$_2$ disrupted the endothelial cell monolayer within 30 minutes after being added to the culture medium, whereas N-Ac-
5   CHGVC-NH$_2$ had no affect on the cells (Figure 7). Endothelial cell morphology was dramatically affected by N-Ac-CHAVC-NH$_2$, and the cells retracted from one another and became non-adherent. These data demonstrate that N-Ac-CHAVC-NH$_2$ is capable of inhibiting endothelial cell adhesion.

Under the same conditions, the cyclic peptides H-CHAVC-NH$_2$, N-Ac-
10 CAHAVDIC-NH$_2$ (Figure 8) and N-Ac-CHAVSC-NH$_2$ had no effect on endothelial cell morphology, indicating that not all cyclic HAV-containing peptides are capable of disrupting endothelial cell adhesion at a concentration of 500µg/mL. It is not unexpected that the potencies of individual cyclic peptides will vary The cyclic peptide (N-Ac-CAHAVDC-NH$_2$ (Figure 9) had a slight effect while N-Ac-CSHAVSSC-NH$_2$
15 (Figure 10) disrupted the endothelial cell monolayer and caused the cells to retract from one another.

<u>EXAMPLE 4</u>

20   <u>Disruption of Human Ovarian Cancer Cell Adhesion</u>

This Example illustrates the use of a representative cyclic peptide to disrupt adhesion of human ovarian cancer cells.

The human ovarian cancer cell line SKOV3 (ATCC #HTB-77) expresses
25 N-cadherin. SKOV3 cells were cultured in a modified MEM–based media containing 10% FCS. Cells were grown in T-250 culture flasks and maintained by periodic subculturing. Cyclic peptides were tested on cells grown in individual wells of 96-well culture dishes (surface area of each well was 0.32cm^2). Cells were harvested from flasks and seeded at a density of 50,000 cells per well in 0.1mL media containing the
30 cyclic peptides at concentrations of 1, 0.1, or 0.01 mg/mL, or in the absence of cyclic peptide. Media control wells were also established. Cultures were evaluated

periodically by microscopic examination under both bright field and phase contrast conditions. Cultures were maintained for 48 hours.

As shown in Figures 11A (compare to Figure 11C) and 12A, the peptide N-Ac-CHAVC-NH$_2$ (final concentration of 1 mg/mL media) disrupted SKOV3 cell

5   adhesion within 24 hours, whereas the control N-Ac-CHGVC-NH$_2$ had no affect on cell adhesion (Figures 11B and 12B). The effect of different amounts of N-Ac-CHAVC-NH$_2$ after 48 hours is shown in Figures 11D-F. In the presence of N-Ac-CHGVC-NH$_2$, (Figures 11B and 12B) the SKOV3 cells formed tightly adherent monolayers. In contrast, the SKOV3 cells did not spread onto the substrata, nor did they form tightly

10   adherent monolayers in the presence of N-Ac-CHAVC-NH$_2$ (Figures 11A, D and 12A). These data demonstrate that N-Ac-CHAVC-NH$_2$ is capable of inhibiting the function of human N-cadherin.

The cyclic peptides N-Ac-CAHAVDIC-NH$_2$, N-Ac-CAHAVDC-NH$_2$ and N-Ac-KHAVD-NH$_2$ were inactive in the SKOV3 cells, indicating that not all cyclic

15   HAV-containing peptides are capable of disrupting epithelial cell adhesion at concentrations of 0.01-1 mg/mL It is not unexpected that the potencies of the cyclic peptides will vary.

20   EXAMPLE 5

                        Disruption of Angiogenesis

Blood vessels are composed of adherent endothelial cells. This Example illustrates the use of a representative cyclic peptide to block angiogenesis (the growth of

25   blood vessels from pre-existing blood vessels).

The chick chorioallantoic membrane assay was used to assess the effects of cyclic peptides on angiogenesis (Iruela-Arispe et al., *Molecular Biology of the Cell* 6:327-343, 1995). Cyclic peptides were embedded in a mesh composed of vitrogen at concentrations of 3, 17, and 33 μg/mesh. The meshes were then applied to 12-day-old

30   chick embryonic chorioallantoic membranes. After 24 hours, the effects of the peptides on angiogenesis were assessed by computer assisted morphometric analysis. The cyclic

peptide H-CHAVC-NH$_2$, with the N-terminal blocking group removed, inhibited angiogenesis by 27%, 34%, and 35% at concentrations of 3, 17, and 33 μg/mesh, respectively. The cyclic peptide N-Ac-CHAVSC-NH$_2$ was found to be inactive in this assay.

5

EXAMPLE 6

Disruption of Normal Rat Kidney (NRK) Cell Adhesion

10   NRK cells express E-cadherin, and monolayer cultures of these cells exhibit a cobblestone morphology. This Example illustrates the ability of a representative cyclic peptide to disrupt NRK cell adhesion.

NRK cells (ATCC #1571-CRL) were plated at 10 – 20,000 cells per 35mm tissue culture flasks containing DMEM with 10% FCS and sub-cultured

15   periodically (Laird et al., *J. Cell Biol. 131*:1193-1203, 1995). Cells were harvested and replated in 35mm tissue culture flasks containing 1 mm coverslips and incubated until 50–65% confluent (24-36 hours). At this time, coverslips were transferred to a 24-well plate, washed once with fresh DMEM and exposed to cyclic peptide solutions (N-Ac-CHAVC-NH$_2$ and N-Ac-CHGVC-NH$_2$) at a concentration of 1mg/mL for 24 hours.

20   Fresh peptide solutions were then added and the cells were left for an additional 24 hours. Cells were fixed with 100% methanol for 10 minutes and then washed three times with PBS. Coverslips were blocked for 1 hour in 2% BSA/PBS and incubated for a further 1 hour in the presence of mouse anti-E-cadherin antibody (Transduction Labs, Lexington, KY; 1:250 dilution). Primary and secondary antibodies were diluted in 2%

25   BSA/PBS. Following incubation in the primary antibody, coverslips were washed three times for 5 minutes each in PBS and incubated for 1 hour with donkey anti-mouse antibody conjugated to fluorescein (Jackson Immuno Research, West Grove, PA; diluted 1:200). Following a further wash in PBS (3 x 5 min) coverslips were mounted and viewed by confocal microscopy.

30   The peptide N-Ac-CHAVC-NH$_2$ disrupted NRK cell adhesion Figure 13D, compare to 13A), whereas N-Ac-CHGVC-NH$_2$ had no affect on cell adhesion

(Figure 13C). In the presence of N-Ac-CHGVC-NH$_2$, the NRK cells formed tightly adherent monolayers with a cobblestone morphology. They also expressed E-cadherin, as judged by immunofluorescent staining protocols (Laird et al., *J. Cell Biol. 131*:1193-1203, 1995) (Figure 14C). In contrast, the NRK cells which were treated with N-Ac-CHAVC-NH$_2$ did not adhere to one another and failed to form a contiguous monolayer (Figure 13D). Furthermore, these cells expressed greatly reduced levels of E-cadherin (Figure 14D). These data demonstrate that N-Ac-CHAVC-NH$_2$ is capable of disrupting NRK cell adhesion.

EXAMPLE 7

Enhancement of Human Skin Permeability

The epithelial cells of the skin (known as keratinocytes) express E-cadherin. This Example illustrates the use of a representative cyclic peptide to enhance the permeability of human skin.

Abdominal skin from humans at autopsy within 24 hours of death was used in these assays. The effect of N-Ac-CHAVC-NH$_2$ and N-Ac-CHGVC-NH$_2$ (used at a concentration of 500 μg/ml) on the penetration of two fluorescent markers, Oregon Green (charge -1, MW 386 daltons) and Rhodamine Green Dextran (no charge, MW 3000 daltons) through human skin was then evaluated utilizing a Franz Cell apparatus (Franz, *Curr. Prob. Dermatol. 7*:58-68, 1978; Franz, *J. Invest. Dermatol. 64*:190-195, 1975). The peptides and markers were dissolved in sterile phosphate buffer, pH 7.2, and phosphate buffer was used as the receptor fluid. The penetration of the markers through the skin was assessed at 6, 12, 24, 36, and 48 hours after the start of the experiment. For each peptide and marker combination, the experiment was performed in triplicate.

N-Ac-CHAVC-NH$_2$ (sample #1) slightly increased the penetration of Oregon Green through the skin, as compared to the effect of N-Ac-CHGVC-NH$_2$ (sample #3) on the penetration of this marker (Table 2). The penetration of Rhodamine

Green through the skin was significantly increased in the presence of N-Ac-CHAVC-NH$_2$, in comparison to N-Ac-CHGVC-NH$_2$ (Table 3).

Table 2

*Percutaneous absorption concentration (μg/5ml) for Oregon Green™ 488
as a function of time

#Sample#	t = 6 hours	t = 12 hours	t = 24 hours	t = 36 hours	t = 48 hours
1Sample#1	0.028	0.096	0.470	0.544	0.665
2Sample#1	0.167	0.322	1.096	1.56	1.725
3Sample#1	0.058	0.352	0.773	0.902	0.971
Mean Sample#1	0.084	0.225	0.780	1.00	1.120
1Sample#3	0.098	0.200	0.709	0.769	0.923
2Sample#3	0.022	0.107	0.864	0.923	1.021
3Sample#3	0.045	0.088	0.522	0.714	0.764
Mean Sample#3	0.055	0.132	0.698	0.802	0.902

* Defined as amount found in the receiver compartment (volume = 5 ml)

Table 3

*Percutaneous absorption concentration (μg/5ml) for
Dextran Rhodamine Green 3000 as a function of time

#Sample#	t = 6 hours	t = 12 hours	t = 24 hours	t = 36 hours	t = 48 hours
1Sample#1	0.4	3.0	16.174	21.044	25.747
2Sample#1	0.8	2.0	4.074	5.556	6.481
3Sample#1	1.2	5.556	13.158	17.565	27.826
Mean Sample#1	0.8	3.52	11.15	14.72	20.02
1Sample#3	0.2	0.6	1.0	1.0	1.8
2Sample#3	0.3	1.0	1.4	1.6	5.370
3Sample#3	0.2	0.4	0.8	1.0	1.8
Mean Sample#3	0.23	0.67	1.07	1.2	2.99

* Defined as amount found in the receiver compartment (volume = 5 ml)

EXAMPLE 8

Disruption of Human Ovarian Cancer Cell Adhesion

5   This Example further illustrates the ability of representative cyclic peptides to disrupt human ovarian cancer cell adhesion.

        The human ovarian cancer cell line OVCAR-3, which expresses E-cadherin, was used in these experiments. Cells were cultured in RPMI supplemented with insulin and containing 20% FCS. Cells were grown in T-250 culture flasks and 10 maintained by periodic subculturing. 'Cells were harvested from flasks and seeded in individual wells of 96-well culture dishes (surface area of each well was $0.32cm^2$) at a density of 50,000 cells per well in 0.1 ml media containing the cyclic peptides (at concentrations of 1, 0.1, or 0.01mg/ml). Media control wells were also established. Cultures were evaluated periodically by microscopic examination under both bright 15 field and phase contrast conditions, and were maintained for 48 hours. N-Ac-CHAVC-NH_2 was found to be inactive within this assay at these concentrations. However, the cyclic peptide N-Ac-CHAVSC-NH_2 disrupted OVCAR-3 adhesion (Figures 15A-C)). This data demonstrates that N-Ac-CHAVSC-NH_2 specifically affects cells that express E-cadherin.

20

EXAMPLE 9

Disruption of Melanoma Cell Adhesion

25   This Example illustrates the ability of a representative cyclic peptide to disrupt melanoma cell adhesion.

        Melanoma ME115 cells (kindly provided by Meenhard Herlyn, Wistar Institute, Philadelphia, PA) were plated on glass coverslips and cultured for 24 hours in 50% keratinocyte growth medium (Clonetics, San Diego, CA) and 50% L15. Fresh 30 medium containing the cyclic peptides (final concentration 500 μg/mL media) N-Ac-CHAVC-NH_2 or N-Ac-CHGVC-NH_2 was then added. Following 24 hours of culture in

the presence of the peptides, the medium was removed and fresh medium containing the peptides was added. The cells were fixed 24 hours later with cold methanol and stored in phosphate buffered saline (PBS).

Coverslips were blocked for 1 hour in 3% ovalbumin/PBS and incubated

5   for a further 1 hour in the presence of rabbit pan-cadherin antibody (Sigma Chemical Co., St. Louis, MO) diluted 1:500. Primary and secondary antibodies were diluted in PBS containing 6% normal goat serum. Following incubation in the primary antibody, coverslips were washed 3 times for 5 minutes each in PBS and incubated for 1 hour in goat anti-rabbit immunoglobulin G conjugated to fluorescein (Kiekegard and Perry,

10   South San Francisco, CA) diluted 1:100. Following a further wash in PBS (3 x 5 minutes) coverslips were mounted in Vectashield (Vector Labs, Burlingame, CA) and viewed with a Zeiss infinity corrected microscope.

Photographs, shown in Figure 16, show an absence of cell membrane staining and the appearance of bright intracellular vesicular staining in cells treated with

15   N-Ac-CHAVC-NH$_2$. In contrast, cells exposed to N-Ac-CHGVC-NH$_2$ displayed cadherin staining all over the cell membrane. Occasionally, the staining concentrated at points of cell-cell contact. These results indicate that the representative cyclic peptide N-Ac-CHAVC-NH$_2$ disrupts melanoma cell adhesion.

20

EXAMPLE 10

Disruption of Breast Cancer Cell Adhesion

This Example illustrates the ability of a representative cyclic peptide to

25   disrupt human breast epithelial cell adhesion.

A1N4 human breast epithelial cells (kindly provided by Martha Stampfer, Lawrence Berkeley Laboratory, Berkeley, CA) were plated on glass coverslips and cultured in F12/DME containing 0.5% FCS and 10 ng/mL EGF for 24 hours. Fresh medium containing the cyclic peptides (final concentration 500μg/mL

30   media) N-Ac-CHAVC-NH$_2$ or N-Ac-CHGVC-NH$_2$ was then added. Following 24 hours of culture in the presence of the peptides, the medium was removed and fresh

medium containing the peptides was added. The cells were fixed 24 hours later with cold methanol and stored in phosphate buffered saline (PBS).

Coverslips were blocked for 1 hour in 3% ovalbumin/PBS and incubated for a further 1 hour in the presence of 1 μg/mL mouse anti-E-cadherin antibody (Zymed,

5   Gaithersburg, MD). Primary and secondary antibodies were diluted in PBS containing 6% normal goat serum. Following incubation in the primary antibody, coverslips were washed 3 times for 5 minutes each in PBS and incubated for 1 hour with goat anti-mouse conjugated to fluorescein (Kiekegard and Perry, South San Francisco, CA) diluted 1:100. Following a further wash in PBS (3 x 5 minutes) coverslips were

10   mounted in Vectashield (Vector Labs, Burlingame, CA) and viewed with a Zeiss infinity corrected microscope.

Photographs, shown in Figures 17 A and B, show reduced E-cadherin staining with a stitched appearance in cells treated with N-Ac-CHAVC-NH$_2$. In addition, holes are present in the monolayer where the cells have retracted from one

15   another. In contrast, cells exposed to N-Ac-CHGVC-NH$_2$ displayed E-cadherin staining concentrated at points of cell-cell contact and formed a tightly adherent monolayer.

20   EXAMPLE 11

Toxicity and Cell Proliferation Studies

This Example illustrates the initial work to evaluate the cytotoxic effects of representative cyclic peptides.

25   N-Ac-CHAVC-NH$_2$ and the control peptide N-Ac-CHGVC-NH$_2$ were evaluated for possible cytotoxic effects on human microvascular endothelial (HMVEC; Clonetics), human umbilical vein endothelial (HUVEC; ATCC #CRL-1730), IAFp2 (human fibroblast cell line; Institute Armand-Frapier, Montreal, Quebec), WI-38 (human fibroblast cell line; ATCC #CCL-75), MDA-MB231 (human breast cancer cell

30   line; ATCC #HTB-26), and PC-3 (human prostate cancer cell line; ATCC #CRL-1435) cells utilizing the MTT assay (Plumb et al., *Cancer Res.* *49*:4435-4440, 1989). Neither

of the peptides was cytotoxic at concentrations up to and including 100 μM. Similarly, neither of the peptides was capable of inhibiting the proliferation of the above cell lines at concentrations up to 100 μM, as judged by ^{3}H-thymidine incorporation assays.

5   In addition, H-<u>CHAVC</u>-NH$_2$ and N-Ac-<u>CHAVSC</u>-NH$_2$, as well as their corresponding control peptides H-<u>CHGVC</u>-NH$_2$ and N-Ac-<u>CHGVSC</u>-NH$_2$ were also evaluated for possible cytotoxic effects on the cell lines outlined above using the MTT assay. Neither of the peptides was cytotoxic at concentrations up to 100 μM. However, N-Ac-<u>CHAVSC</u>-NH$_2$ and N-Ac-<u>CHGVSC</u>-NH$_2$ inhibited the proliferation of HUVEC at concentrations (IC$_{50}$ values) of 57 μM and 42 μM respectively, as judged by ^{3}H-
10   thymidine incorporation assays. N-Ac-<u>CHAVSC</u>-NH$_2$ also inhibited the proliferation of MDA-MB231 cells at a concentration of 52 μM (Tables 4 and 5).

Table 4

Evaluation of Peptides for Cytotoxicity and Capacity to Inhibit Cell Proliferation
of Normal Cells (IC$_5$0 in mM)

5

Peptide	Normal Cells							
	HMVEC		HUVEC		IAFp2		WI-38	
	Cell prol	Cytotox	Cell Prol	Cytotox	Cell Prol	Cytotox	Cell Prol	Cytotox
N-Ac-CHGVC-NH$_2$ (control for #1)	>100mM	>100mM	>100mM	>100mM	>100mM	>100mM	>100mM	>100mM
N-Ac-CHAVC-NH$_2$ (#1)	>100mM	>100mM	>100mM	>100mM	>100mM	>100mM	>100mM	>100mM
H-CHGVC-NH$_2$ (control for #2)	>100mM	>100mM	>100mM	>100mM	>100mM	>100mM	>100mM	>100mM
H-CHAVC-NH$_2$ (#2)	>100mM	>100mM	>100mM	>100mM	>100mM	>100mM	>100mM	>100mM
N-Ac-CHGVSC-NH$_2$ (control for #18)	>100mM	>100mM	42mM	>100mM	>100mM	>100mM	>100mM	>100mM
N-Ac-CHAVSC-NH$_2$ (#18)	>100mM	>100mM	57mM	>100mM	>100mM	>100mM	>100mM	>100mM

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Table 5

Evaluation of Peptides for Cytotoxicity and Capacity to Inhibit Cell Proliferation
of Tumoral Cells (IC_5O in mM)

Peptide	Tumoral Cells			
	MDA-	MB231	PC-3	
	Cell Prol	Cytotox	Cell Prol	Cytotox
N-Ac-CHGVC-NH$_2$ (control for #1)	>100mM	>100mM	>100mM	>100mM
N-Ac-CHAVC-NH$_2$ (#1)	>100mM	>100mM	>100mM	>100mM
H-CHGVC-NH$_2$ (control for #2)	>100mM	>100mM	>100mM	>100mM
H-CHAVC-NH$_2$ (#2)	>100mM	>100mM	>100mM	>100mM
N-Ac-CHGVSC-NH$_2$ (control for #18)	>100mM	>100mM	>100mM	>100mM
N-Ac-CHAVSC-NH$_2$ (#18)	52mM	>100mM	>100mM	>100mM

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From the foregoing, it will be evident that although specific
embodiments of the invention have been described herein for the purpose of illustrating
the invention, various modifications may be made without deviating from the spirit and
10   scope of the invention.